# NAXIVA

## PHASE II NEOADJUVANT STUDY OF AXITINIB FOR REDUCING EXTENT OF VENOUS TUMOUR THROMBUS IN CLEAR CELL RENAL CELL CANCER WITH VENOUS INVASION (NAXIVA)

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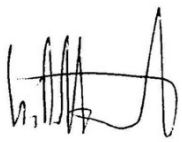
Signature:  Date: 22/09/2017

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**SYNOPSIS**

| | |
|---|---|
| Protocol ID | NAXIVA |
| Protocol Title | Phase II **N**eoadjuvant study of **AXI**tinib for reducing extent of venous tumour thrombus in clear cell renal cell cancer with **V**enous inv**A**sion (NAXIVA) |
| Development Phase | Phase II Feasibility |
| Primary Endpoint | The percentage of evaluable patients with an improvement in the Mayo classification.<br><br>Mayo Classification;<br>• Level 0: thrombus limited to the renal vein<br>• Level 1: into IVC <2cm from renal vein ostium level<br>• Level 2: IVC extension >2cm from renal vein ostium and below hepatic vein<br>• Level 3: thrombus at the level of or above the hepatic veins but below the diaphragm<br>• Level 4: thrombus extending above the diaphragm. |
| Secondary Endpoints | % change in surgical approach.<br>% change in venous tumour thrombus (VTT) height.<br>Response rate (RECIST).<br>Evaluation of morbidity assessed by Clavian-Dindo classification. |
| Study Design | NAXIVA is a single arm, single agent, open label, phase 2 feasibility study of axitinib in patients with both metastatic and non-metastatic renal cell carcinoma of clear cell histology prior to nephrectomy and thrombectomy. |
| Patient Accrual | 20 patients will be recruited over a 24 month period at 7 sites across the UK. |
| Interim Analysis | Will be performed after thirteen patients have been recruited. If no patients show an improvement in their Mayo classification the trial will be stopped for futility. |

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**GLOSSARY OF ABBREVIATIONS**

| | |
|---|---|
| AE | Adverse Event |
| AIDS | Acquired Immunodeficiency Syndrome |
| ALT | Alanine Transaminase |
| AR | Adverse Reaction |
| AST | Aspartate Transaminase |
| BID | Twice a day |
| BP | Blood Pressure |
| ccRCC | Clear Cell Renal Cell Cancer |
| CI | Chief Investigator |
| CRF | Case Report Form |
| CRP | C-Reactive Protein |
| CRUK | Cancer Research United Kingdom |
| CSA | Common Services Agency |
| CT | Computed Tomography |
| CTCAE | Common Toxicity Criteria for Adverse Events |
| ct DNA | Circulating Tumour DNA |
| DCE | Dynamic Contrast Enhanced |
| DMC | Data Monitoring Committee |
| DWI | Diffusion Coefficient |
| EDTA | Ethlenediaminetetraacetic Acid |
| EMT | Epithelial Mesenchymal Transition |
| EU | European Union |
| GCP | Good Clinical Practice |
| GP | General Practitioner |
| HIV | Human Immunodeficiency Virus |
| ICH | International Conference on Harmonisation |
| IC50 | 50% Inhibitory Concentration |
| IMP | Investigational Medicinal Product |
| IVC | Inferior Vena Cava |
| LDH | Lactate Dehydrogenase |
| M0 | Non-metastatic |
| M1 | Metastatic |
| MHRA | Medicines and Healthcare products Regulatory Agency |
| mRCC | Metastatic Renal Cell Carcinoma |
| MRI | Magnetic Resonance Imaging |
| MSKCC | Memorial Sloan Kettering Cancer Centre |
| NSCLC | Non Small Cell Lung Cancer |
| ORR | Objective Response Rate |
| PBS | Phosphate Buffered Saline |
| PCR | Protein: Creatinine Ratio |
| PDGF-R | Platelet-Derived Growth Factor Receptors |
| PI | Principal Investigator |
| PIL | Patient Information Leaflet |
| PMBC | Peripheral Blood Mononuclear Cells |
| PRES | Posterior Reversible Encephalopathy Syndrome |
| PSA | Prostrate-specific antigen |
| QP | Qualified Person |
| RCC | Renal Cell Cancer |
| R&D | Research and Development |
| REC | Research Ethics Committee |
| RECIST | Response Evaluation Criteria in Solid Tumours |
| SAE | Serious Adverse Event |
| SCTRU | Scottish Clinical Trials Research Unit |

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|---|---|
| SDV | Source Data Verification |
| SmPC | Summary of Product Characteristics |
| SOP | Standard Operating Procedures |
| SUSAR | Suspected Unexpected Serious Adverse Reaction |
| TKI | Tyrosine Kinase Inhibitor |
| TMG | Trial Management Group |
| TSC | Trial Steering Committee |
| TSH | Thyroid Stimulating Hormone |
| TTP | Thrombotic Thrombocytopenic Purpura |
| TV | Tumour Thrombus Volume |
| UKCRC | United Kingdom Clinical Research Collaboration |
| ULN | Upper Limits of Normal |
| VEGF | Vascular Endothelial Growth Factor |
| VEGFR | Vascular Endothelial Growth Factor Receptor |
| VTT | Venous Tumour Thrombus |

# 1. INTRODUCTION

## 1.1. Background

Venous tumour thrombus (VTT) extension into the renal vein and/or inferior vena cava (IVC) occurs in 4-15% cases of renal cell cancer (RCC) (1). If the patient is fit enough to undergo surgery, in an attempt to excise all disease with curative intent, the surgical risk is high due to the need to perform a cavotomy and/or open heart surgery to excise the VTT (5-15% mortality) (1). Complications are established to increase with the height of the VTT (1). After such surgery, the 5 year survival rates are poor; ~40-65% in non-metastatic RCC to 0-17% for patients with concomitant metastatic RCC (2, 3). As such, the concept of using targeted therapies, which are standard of care in metastatic RCC, to downstage the VTT prior to extirpative surgery is appealing. If it were possible to reduce the extent of the surgery an individual patient with RCC VTT required it is likely morbidity and mortality would be reduced and potentially survival time improved.

## 1.2. Investigational Medicinal Product

Axitinib is a potent oral VEGFR2 and 3 inhibitor at picomolar concentrations and VEGFR1, PDGFRs and c-KIT inhibitor at low nanomolar concentrations. In phase II clinical trials, axitinib has shown efficacy in sorafenib and cytokine refractory mRCC patients. A recently reported phase III trial, showed superiority over sorafenib as second line therapy (4), leading to the licensing of axitinib in this indication by the United States Food and Drug Administration (FDA) agency in January 2012. Axitinib was subsequently licensed for the same indication by the European Medicines Agency. Furthermore, the efficacy of axitinib is under evaluation in other tumour types, and has shown activity in lung (5), thyroid (6), and pancreatic (7) cancers, and melanoma (8).

## 1.3. Pre-Clinical Data

In vitro, axitinib inhibits cellular phosphorylation of VEGFR2 and 3 with an IC50 of about 0.2 nmol/L. It has a higher IC50 for VEGFR1 (1.2 nmol/L), PDGFR-β (1.6 nmol/L), PDGFR-α (5 nmol/L) and c-KIT (1.7 nmol/L). It reduces phosphorylation of downstream signalling molecules mediated by vascular endothelial growth factor (VEGF) in a rapid and dose-dependent manner, including Akt, endothelial nitric oxide synthase and extracellular signal regulated kinase (ERK).
In mouse xenografts, twice daily oral axitinib inhibited the primary tumour and controlled metastases in human melanoma (M24mwt), colorectal cancer (HCT-116) and RCC (SN12C) models. Within axitinib treated tumours, there was reduced CD31 and Ki-67 staining and increased caspase-3 staining. Microscopic examination of tumour vasculature in preclinical models of pancreatic islet cell tumours and Lewis lung carcinomas has shown that axitinib reduces patency and flow. Axitinib has its greatest effects on endothelial cells and fenestrated vessels, resulting in normalisation of the surviving tumour vasculature.

## 1.4. Clinical Data

### 1.4.1 Efficacy Data

**Phase I**
In an initial phase I study, 3 of 36 patients had partial responses determined by Response Evaluation Criteria in Solid Tumours (RECIST) (9) including 2 patients with RCC and 1 patient with adenoid cystic carcinoma (10). In a Japanese phase I trial, none of 12 recruited patients had a partial response but 3 patients had stable disease at 24 weeks, seen in one patient each with colorectal carcinoma, thymic cancer and non-small cell lung cancer (NSCLC) (11).

**Phase II: Axitinib in Cytokine-refractory Metastatic RCC**
In a single arm, open label, phase II trial in cytokine-refractory mRCC, axitinib was administered at a starting dose of 5mg twice daily in the fasted state as 28 day treatment cycles until disease progression or significant toxicity (12). Fifty-two patients were recruited with a median age of 59 years and performance status 0 or 1. Forty-nine patients (95%) had previously undergone a nephrectomy and none of the patients had previously received tyrosine kinase inhibitors (TKIs). All patients had clear cell histology, except one patient who had papillary carcinoma. The objective response rate (ORR) was 44.2% (95% CI: 30.5-58.7%); there were 2 complete and 21 partial responses. Twenty-two patients had stable disease for at least 8 weeks, including the patient with papillary carcinoma. Four patients had early progression and 3 patients could not be assessed for response. The median thrombotic thrombocytopenic purpura (TTP) was 15.7 months and median overall survival was 29.9 months. The median duration of axitinib therapy was 9.4 months and median dose was 8.83 mg/day, as 15 patients had dose reductions for adverse events (AE) including fatigue, hypertension and diarrhoea. Axitinib was discontinued due to adverse events in ten patients. The most frequent grade 1-4 axitinib related adverse events were diarrhoea, hypertension, fatigue, nausea and hoarseness. The most common grade 3-4 adverse events were hypertension (15.4%), diarrhoea (9.6%) and fatigue (7.7%). Thirty patients had axitinib induced hypertension, eight of whom had grade 3-4 hypertension. Hypertension was resistant to anti-hypertensive therapy in 8 patients, seven of whom had hypertension at baseline. There were no detectable haematological toxicities. Four patients had treatment related proteinuria which resolved once axitinib was stopped.

**Phase II: Axitinib in Sorafenib Refractory Metastatic RCC**
In a single arm, open label, phase II study, patients with sorafenib refractory mRCC received a starting dose of axitinib 5mg twice daily with food until disease progression or unmanageable toxicity (13). Sixty-two patients were recruited; all had undergone prior nephrectomy and 59 patients had clear cell /mixed histology. Median age was 60 years and all patients were performance score of 0 or 1. The most common prior therapies in addition to sorafenib were sunitinib and cytokine therapy, received by 14 (22.6%) and 38 (61.3%) patients, respectively.
The ORR was 22.6% (95% CI 12.9-35.0%), with 14 patients experiencing a partial response and no complete responses. Tumour responses were observed in patients who received 5 mg twice daily or higher, and also in patients whose dose was reduced to below 5 mg twice daily. Patients who had a partial response received doses ranging from <4mg to 9-10mg twice daily. Eleven patients (17.8%) had stable disease. The median progression-free survival was 7.4 months (95% CI 6.7-11.0 months) and median overall survival was 13.6 months (95% CI 8.4-18.8 months).
The most common grade 3-4 adverse events included hypertension (16.1%), fatigue (16.1%), hand-foot syndrome (16.1%), dyspnoea (14.5%) and diarrhoea (14.5%). Twelve patients stopped axitinib due to treatment related adverse events. Two patients developed congestive heart failure, both of whom had a history of cardiovascular disease, and two patients had cerebral haemorrhages during the study. One of the latter patients was subsequently diagnosed with a cerebral metastasis at the site of haemorrhage. Most haematological toxicities were mild or moderate (grade 1 or 2) except grade 3 lymphopaenia experienced by 9 of 55 evaluable patients (16.4%).

### 1.4.2 Safety Data

**Phase I**
The primary dose limiting toxicity in the initial phase I trial was hypertension (10); in most cases it responded to anti-hypertensive therapy and resolved after axitinib was stopped. The incidence and severity of hypertension was dose-dependent, all patients with axitinib-

induced hypertension at dose 5mg twice daily were managed with standard anti-hypertensives. Prior to blood pressure monitoring two patients had uncomplicated seizures in the absence of brain metastases at doses 10mg and 20mg twice daily, these may have been related to hypertensive crises.
Three bleeding events were reported during the phase I study. A patient with central NSCLC had a fatal haemoptysis attributed to axitinib. A patient with peripheral NSCLC had grade 1 haemoptysis while taking axitinib which was subsequently stopped. Two weeks later the patient had grade 4 haemoptysis and died, this patient's death was reported as secondary to disease progression and concurrent infection. Finally, there was 1 episode of grade 1 rectal bleeding.
Asymptomatic proteinuria was detected in seven of the first ten patients. Consequently, patients with proteinuria >0.5gram/24 hours were not recruited and treatment reviews were required for all patients with proteinuria ≥1gram/24 hours. These amendments reduced the incidence and severity of proteinuria.

Thrombocytopenia was the only haematological toxicity in the phase I trial, with grade 2 thrombocytopenia affecting one patient taking 20mg twice daily.

**Phase II**
The commonest non-haematological adverse events grade 1-4 reported in phase II axitinib monotherapy trials were hypertension, fatigue, diarrhoea, anorexia, nausea and hoarseness. The main grade 3-4 adverse events were similar, except hand-foot syndrome and proteinuria were also reported. Most adverse events were manageable. Hypertension usually responded to anti-hypertensive therapy and resolved once axitinib was stopped.
The starting dose in all but one of the phase 2 studies conducted to date was 5mg twice daily of axitinib. In one metastatic breast cancer study, axitinib dose was titrated up from a starting dose of 5mg twice daily in 1–3mg increments in patients tolerating axitinib. Those subjects who could tolerate axitinib with no adverse events related to axitinib above CTCAE (14) grade 2 for consecutive 2 week periods were permitted to increase their dose step-wise to 7mg twice daily and then to 10mg twice daily, unless their BP was >150/90mm Hg or the subject was receiving antihypertensive medication. All studies ongoing at the time of writing allow axitinib dose reductions to as low as 2mg twice daily for treatment-related adverse events. Except for an increase in hand-foot syndrome and slight increase in the incidence of hypertension, it appears that patients whose dose is titrated to between 6-10mg twice daily doses do not experience increased toxicities if they have previously tolerated 5mg twice daily starting dose. Axitinib dose titration will be permitted within NAXIVA, consistent with previous clinical trial protocols and the drug development programme for axitinib.

### 1.5. Trial Rationale

There is no level I or II evidence of neoadjuvant studies of targeted therapies in non-metastatic RCC VTT or upfront therapies in metastatic RCC VTT. There is level III evidence of mixed populations of RCC VTT patients (including both metastatic and non-metastatic patients) treated with a heterogeneous group of drug treatments (15, 16). The results of these studies suggest that regression in VTT is limited to sunitinib therapy (seen in 3/12=25% patients; regression not seen for bevacizumab, temsirolimus or sorafanib). With sunitinib treatment none of the patients (0/12) had an increase in the VTT level (15).

A recent prospective phase II of 12 weeks neoadjuvant axitinib in ccRCC T2-T3b, has been reported by Karam and colleagues (17). All of the patients in this study were cT3a (i.e. there were no patients with VTT). It was possible to titrate all patients up to 10mg axitinib with no grade 4/5 toxicities. 100% of patient's tumours showed response (46% patients had partial response, and 54% stable disease) and the median reduction in primary tumour diameter was 28% by week 12. The median tumour diameter reduced from 10cm to 6.9cm. The vast majority of reduction in tumour size had occurred at 7 weeks of axitinib treatment.

The results of these small studies in non-metastatic RCC patients suggest that neoadjuvant TKI treatment of RCC patients is safe. However, the effect of these drugs on the extent of the VTT and the surgical approach that must be applied has not been confirmed. Authors in this field agree that a prospective trial is required to answer this important clinical question (15,16,18).

In NAXIVA we will study the response of VTT to axitinib. The primary outcome is percentage of patients with reduction in the Mayo Classification (19). This study will address the feasibility of patient recruitment in this setting. In order to ensure adequate recruitment both metastatic and non-metastatic patients will be recruited to assess the effect of axitinib therapy on the extent of the tumour thrombus, rather than to primarily assess a prolonged survival. A reduction in the extent of the VTT, as assessed by the Mayo classification, will potentially result in less extensive and less morbid surgical approach with immediate patient benefits of reduced operative mortality/morbidity, potential shorter hospital stay and shorter recuperation period to return to full activities of daily living. There is also potential for a longer disease free survival in patients treated with axitinib prior to nephrectomy and tumour thrombectomy. The risks of the treatment of patients with VTT with axitinib over an 8 week period include progression of their disease (from operable to non-operable or non-metastatic to metastatic). However, based on the studies outlined above there is no evidence that progressive disease will occur over this period of time. Treating patients with axitinib over an 8 week period may also increase the incidence of adverse events which could have a significant impact on the patient's fitness for surgery. This risk will be minimised as dose reduction and axitinib cessation will be managed as per section 4.

Axitinib has been chosen for this study as it is a potent TKI with proven effect in non-metastatic ccRCC (17). The dose regimen (starting at 5mg bd, increasing dose up to 10mg twice daily (BID), as rapidly as possible, as tolerated by patients) is a well established regimen in metastatic ccRCC and was shown to be effective in the previous non-metastatic ccRCC neoadjuvant study (18). 8 weeks has been chosen as the treatment duration as in the study by Karam et al maximum effect on the primary tumour size was observed by 7 weeks and no significant further advantage was seen by continuing treatment to 12 weeks.

Patients having biopsy proven ccRCC will be included in this study, as this is histological subtype of RCC shown to have a benefit from axitinib therapy. Patients with VTT confined to the renal vein (cT3a) will be included as they would benefit from a reduction in the extent of the VTT, regressing from the renal vein back into the kidney, as this would allow curative surgery by laparoscopic rather than more morbid open surgical approaches. Patients with more extensive IVC VTT (infrahepatic, intrahepatic or atrial) will be included, as for patients in each of these groups reducing the extent of the thrombus i.e. from intrahepatic to infrahepatic would potentially reduce extent of surgery and the associated surgical morbidity. Both non-metastatic and metastatic patients will be eligible for recruitment as both groups of patients are likely to benefit, in terms of reduction of level of VTT and surgical morbidity, if axitinib shows efficacy in this patient cohort. It is accepted that there may be different biological processes at play in these 2 cohorts. However, this will be further explored in a fully powered phase 3 randomised control trial, if this phase 2 trial is positive.

## 2. TRIAL OBJECTIVES

**Aims:** to assess the reduction in the Mayo Venous Tumour Thrombus Classification of patients with clear cell renal cell cancer who have a renal vein or inferior vena cava venous tumour thrombus treated with neoadjuvant (non-metastatic patients) or upfront (metastatic patients) axitinib therapy.

**Primary outcomes:**

- The percentage of evaluable patients with an improvement in the Mayo Classification.

Mayo Classification;

- Level 0: thrombus limited to the renal vein
- Level 1: into IVC <2cm from renal vein ostium level
- Level 2: IVC extension >2cm from renal vein ostium and below hepatic vein
- Level 3: thrombus at the level of or above the hepatic veins but below the diaphragm
- Level 4: thrombus extending above the diaphragm.

**Secondary outcomes:**

- % change in surgical approach
- % change in VTT height
- Response rate (RECIST)
- Evaluation of surgical morbidity assessed by Clavian-Dindo classification

## 3. TRIAL DESIGN

### 3.1. General Design

NAXIVA is a single arm, single agent, open label, phase II feasibility study of axitinib in patients with both metastatic and non-metastatic renal cell carcinoma of clear cell histology. 20 patients will be recruited from multiple centres within the United Kingdom.

Patients who have signed informed consent and who have met all eligibility criteria will be registered into the trial.

The starting dose of axitinib will be 5mg BID and escalated to 7mg BID and then 10mg BID. A dose modification assessment will take place every 2 weeks in clinic during the 8 week pre-surgical treatment period and will be dependent on tolerability of treatment. Patients will follow an aggressive axitinib dose escalation process within the 8 week period to a maximum of 10mg BID. Patients should stop axitinib a minimum of 36 hours and a maximum of 7 days prior to surgery in week 9.

Blood, urine and tissue samples will be taken prior to and during therapy to evaluate biomarkers of treatment response. Nephrectomy and IVC tumour thrombectomy will be planned for all patients on the trial.
Response to axitinib in VTT, primary tumour and any RECIST measureable lesion will be correlated with changes in molecular markers.

Patients will be followed up in clinic at 6 & 12 weeks post surgery.

### 3.2. Inclusion Criteria

1. Age ≥ 18.
2. Histologically proven clear cell RCC.
3. Immediate resection of the primary tumour considered technically possible.

4. Suitable for and willing to undergo nephrectomy (either cytoreductive or with curative intent)
5. cT3b, cT3c, cT3a (main renal vein)
6. N0, N1, or Nx
7. M0, or M1
8. ECOG performance status 0 – 1
9. Urinalysis <2+ protein. If dipstick is ≥2+ then a 24-hour urine collection should be performed and the patient may enter NAXIVA only if urinary protein is <2g per 24 hours.
10. All female patients with reproductive potential must have a negative serum or urine pregnancy test within a maximum of 14 days prior to starting trial treatment.

**3.3. Exclusion Criteria**

1. For M1 patients: poor risk on Memorial Sloan Kettering Cancer Centre (MSKCC) score and deemed suitable for cytoreductive nephrectomy at time of enrolment.
2. The presence of active second malignancy. Patients will be eligible if they have adequately treated basal cell carcinoma, squamous cell skin cancer, in situ cervical cancer, stable prostate cancer or if treated with curative intent for any other cancer with no evidence of disease for 2 years. Patients with prostate cancer will be permitted entry if not receiving treatment and prostrate-specific antigen (PSA) is not rising.
3. Women who are pregnant or are breastfeeding. Female patients must be surgically sterile, be postmenopausal, or must agree to use effective contraception during the period of therapy and up to 1 week after treatment.
   Male patients must be surgically sterile or must agree to use effective contraception during the period of therapy and for 6 months after completion of study drug (Patients who do not meet this will not be are not eligible).
4. Current signs or symptoms of severe progressive or uncontrolled hepatic, endocrine or pulmonary disease other than directly related to RCC.
5. Gastrointestinal abnormalities including: a. inability to take oral medication; b. requirement for intravenous alimentation; c. prior surgical procedures affecting absorption including total gastric resection; d. treatment for active peptic ulcer disease in the past 6 months; e. active gastrointestinal bleeding, unrelated to cancer, as evidenced by hematemesis, hematochezia or melena in the past 3 months without evidence of resolution documented by endoscopy or colonoscopy; f. malabsorption syndromes.
6. Current use or anticipated need for treatment with drugs that are known potent CYP3A4 inhibitors (see section 4.4, concomitant therapy).
7. Current use, or anticipated need for treatment with, drugs that are known CYP3A4 inducers or substrates for CYP1A2 (see section 4.4, concomitant therapy).
8. Requirement of anticoagulant therapy with oral vitamin K antagonists. Low-dose anticoagulants for maintenance of patency of central venous access device or prevention of deep venous thrombosis is allowed. Therapeutic use of low molecular weight heparin is allowed.
9. Active seizure disorder, spinal cord compression, or carcinomatous meningitis.
10. Any of the following within 12 months prior to study entry: myocardial infarction, uncontrolled angina, coronary/peripheral artery bypass graft, symptomatic congestive heart failure, cerebrovascular accident or transient ischemic attack.
11. Uncontrolled hypertension (>160/100 mmHg despite optimised antihypertensive treatment).
12. Known human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS)-related illness.
13. ALT or AST ≥ 1.5 x ULN; Bilirubin ≥ 1.5 x ULN.
14. Serum creatinine ≥ 1.5 x ULN

15. Neutrophil count < 1.0 x $10^9$/L; platelet count < 100 x $10^9$/L; Hb ≤ 90g/L.
16. Known severe hepatic impairment (Child-Pugh class C)
17. Known hypersensitivity to axitinib or any of its excipients. Specifically patients with hereditary galactose intolerance, Lapp lactase deficiency or glucose-galactose malabsorption should not enter the study.

### 3.4. Endpoints

**Primary**:

- The percentage of evaluable patients with an improvement in the Mayo Classification.

Mayo Classification;

- Level 0: thrombus limited to the renal vein
- Level 1: into IVC <2cm from renal vein ostium level
- Level 2: IVC extension >2cm from renal vein ostium and below hepatic vein
- Level 3: thrombus at the level of or above the hepatic veins but below the diaphragm
- Level 4: thrombus extending above the diaphragm.

**Secondary**:

- % change in surgical approach
- % change in VTT height
- Radiological response (RECIST)
- Evaluation of morbidity assessed by Clavian-Dindo classification

**Primary Safety Endpoints**

Thirteen patients will be recruited at first; if at least 1 patient has an improvement in the Mayo classification a further 7 patients would be recruited (to the final total of 20 patients who have received study drug), to receive study drug. At least 3 patients showing reductions in Mayo score are required for a positive trial. The early stopping rules are:

(1) If a single M0 patient progresses to the point where surgery is no longer possible, then recruitment of new patients will be suspended following a review by the data monitoring committee (DMC).
(2) If a single M0 patient becomes M1 recruitment of new patients will be suspended following a review by the DMC.
(3) If in 3 patients the VTT extends but patients remain surgically resectable the trial will close. If the VTT has extended the patient will be expedited and operated on as soon as possible.

Where there is diagnostic doubt at site, the week 3 MRI abdomen scans (and CT chest for M0 patients) a central review will be conducted to determine if a patient has progressed as per RECIST 1.1 criteria. In addition those particular patient cases will be discussed on an individual basis by the trial steering committee (TSC).
The central review for final analysis will be conducted by radiologists based at Addenbrooke's Hospital.

## 4. TREATMENT

### 4.1. Treatment Schedule

Patients should start axitinib treatment on day 1, week 1 of the study and continue for 8 weeks.
Patients will be prescribed 1 pack (28 tablets) of 5mg BID at week 1. Patients will then be prescribed further axitinib every 2 weeks following the dose modification schedule detailed in

section 4.2 (depending on toxicities and blood pressure). Doses should be taken appropriately 12 hours apart and patients should be instructed to take their doses at approximately the same time each day with or without food as per instruction. On clinic days only, patients will be advised to fast for 6 hours prior to their clinic visit.
Patients should be advised to stop axitinib treatment a minimum of 36 hours and maximum of 7 days prior to week 9 surgery and tissue collection.
If a patient vomits or misses a dose, an additional dose should not be taken. The next prescribed dose should be taken at the usual time. If the patient reports having missed a dose or vomiting, this should be recorded in the source documents and case report forms (CRFs).

### 4.2. Dose Modifications

| Dose Level | Dose | Dispensed As |
|---|---|---|
| **+2** | **10mg BID** | **2 x 5mg tablets BID (2 x 5mg packs)** |
| **+1** | **7mg BID** | **1 x 5mg tablet BID + 2 x 1mg tablets BID (1x 5mg + 2 x 1mg packs)** |
| **0 (starting dose)** | **5mg BID** | **1 x 5mg tablet BID (1 x 5mg pack)** |
| **-1** | **3mg BID** | **3 x 1mg tablets BID (3 x 1mg packs)** |
| **-2** | **2mg BID** | **2 x 1mg tablets BID (2 x 1mg packs)** |

**Dose Escalation**
Patients who tolerate axitinib with no adverse events related to study drug above CTCAE grade 2 for a consecutive 2 week period should follow the aggressive dose escalation schedule above and have their dose increased by one dose level to a maximum of 10 mg BID. The only exception to this would be if the patient's blood pressure (BP) is >150/90 mm Hg or the patient is receiving antihypertensive medication. If the patient is receiving antihypertensive medication and a dose escalation is clinically indicated this should be considered by the onsite clinical team.

**Dose Interruption and Reduction**
Patients experiencing non-haematological drug reactions greater than CTCAE Grade 2 or haematological reactions greater than CTCAE Grade 3 should undergo dose modification. The current version of the Summary of Product Characteristics (SmPC) should be used to assess whether any adverse event is attributable to the drug.
Axitinib should be stopped in the event of significant toxicity and restarted if appropriate when toxicities have resolved. If the patient has had an interruption of axitinib more than 2 weeks, it must be discussed with the Chief Investigator (CI) before the patient restarts study drug.
Patients permanently removed from treatment for intolerable toxicity should receive a post treatment CT scan assessed according to RECIST 1.1 criteria, and should revert to standard of care.

The criteria for dose modification for axitinib related adverse events are summarised in the table below:

**Criteria for dose modification for axitinib- related adverse events (other than hypertension or proteinuria)**

| Related Adverse Events | Intervention |
|---|---|
| Any grade: bleeding where medical | Temporarily interrupt the axitinib dose. |

| | |
|---|---|
| intervention is required | |
| Any grade: posterior reversible encephalopathy syndrome (PRES) | Patients with signs or symptoms should temporarily interrupt or permanently discontinue axitinib treatment. In case of severe or persistent arterial hypertension and symptoms suggestive of posterior reversible encephalopathy syndrome, a diagnostic brain magnetic resonance image (MRI) should be considered. If PRES is confirmed, the patient should permanently discontinue axitinib. |
| Grade 1 | Continue at same dose level |
| Grade 2 | Continue at same dose level |
| Grade ≥3 non-haematologic treatment-related toxicity or Grade 4 haematologic toxicity (other than those below) | Interrupt dosing; re-start at one lower dose level as soon as improvement to CTCAE Grade≤2. If patient requires dose reduction below 2mg BID, contact SCTRU for discussion prior to implementation. |
| Grade 4 lymphopaenia | Continue at the same dose level at the discretion of the investigator. |

**Axitinib dose reduction guidance for hypertension**

| **Degree of Blood Pressure Elevation** | | | **Management** |
|---|---|---|---|
| Systolic Pressure | | Diastolic Pressure | |
| 2 consecutive BP readings show systolic pressure >150 mmHg | OR | 2 BP readings separated by at least 1 hour show diastolic pressure >90 mmHg | If not on maximal antihypertensive treatment, institute new or additional antihypertensive medication and maintain dose of axitinib. If on maximal antihypertensive treatment, reduce axitinib to one lower dose level. |
| 2 consecutive BP readings show systolic pressure >160 mmHg | OR | 2 BP readings separated by at least 1 hour show diastolic >100 mmHg | Interrupt dosing *; adjust antihypertensive medication; as soon as BP is less than 150/90 mmHg, restart axitinib at one lower dose level. |
| Recurrent hypertension following previous dose reduction (2 consectutive BP readings show systolic pressure >150 mmHg) | OR | Recurrent dBP>90 mmHg (2 BP readings separated by at least 1 hour) following previous dose reduction. | Repeat axitinib dose reduction by one lower dose level. If a patient requires dose reduction below 2 mg BID, contact SCTRU for discussion. |

* If dose is interrupted, patients receiving antihypertensive medications should monitor closely for hypotension.

**Axitinib Dose reduction for Proteinuria**

- If dipstick shows ≥2 proteinuria, perform 24 hour urine collection or urinary protein:creatinine ratio (PCR). Dosing may continue while waiting for test results.
- If < 2g proteinuria/24 hour or urinary PCR < 200mg/mmol is reported, continue dosing at the same dose level.
- If ≥ 2g proteinuria/24 hours or urinary PCR ≥ 200mg/mmol is reported, withhold dose and repeat 24 hour urine collection or urinary PCR (interval at investigator discretion) until proteinuria is < 2 g/24 hours or urinary PCR < 200mg/mmol. Restart axitinib at

the same dose or one lower dose level at discretion of the investigator. Monitor renal function in accordance with standard practice.

**Dose re-escalation**
Re-escalation back to the previous dose level is permitted in the absence of grade ≥3 haematologic or grade ≥2 non-haematologic treatment-related toxicity in the previous 4 weeks of treatment.

**Axitinib dose interruption for surgery or surgical procedures**
If major surgery or an interventional procedure (e.g. endoscopy) which is not part of NAXIVA study is required, treatment with axitinib must be interrupted a minimum of 36 hours and a maximum of 7 days before the procedure and the patient's blood pressure should be monitored closely for hypotension. Patients may resume axitinib seven days after minor surgery and 2-3 weeks after major surgery, assuming the wounds have completely healed. The decision to resume axitinib therapy after surgery should be based on clinical judgment of adequate wound healing.

### 4.3. Duration of Treatment

Treatment with study drug should be discontinued if it is considered to be in the best interest of the patient. Reasons for treatment discontinuation include:

- Disease progression
- Occurrence of intolerable side effects
- Pregnancy
- Patient withdrawal of consent or non-compliance

### 4.4. Concomitant Therapy

Axitinib is metabolised primarily by liver enzymes, in particular CYP3A4. All medication considered necessary for the patients' welfare and which is not expected to interfere with the evaluation of the study drug may be given at the discretion of the investigator. Concomitant medications must be recorded in the patients' source documentation, as well as the appropriate pages of the CRF.

Contraindicated concurrent medications include:

| Agents known to induce CYP3A4 or CYP1A2 including but not limited to: | Agents known to inhibit CYP1A2, CYP2C19 or CYP3A4 including but not limited to: |
|---|---|
| o Amobarbital,<br>o Carbamazepine,<br>o Dexamethasone,<br>o Felbamate,<br>o Nevirapine,<br>o Omeprazole,<br>o Phenobarbital,<br>o Phenytoin,<br>o Primidone,<br>o Rifabutin,<br>o Rifampicin,<br>o St John's wort | o Amitriptyline<br>o Amprenavir,<br>o Artemisinin,<br>o Atazanavir,<br>o Atazanavir,<br>o Cimetidine,<br>o Ciprofloxacin,<br>o Clarithromycin,<br>o Delavirdine<br>o Enoxacin,<br>o Erythromycin,<br>o Ethinyl,<br>o Fluvoxamine,<br>o Fosamprenavir<br>o Grapefruit juice,<br>o Imipramine<br>o Indinavir, |

| | |
|---|---|
| | ○ Itraconazole,<br>○ Ketoconazole,<br>○ Lopinavir,<br>○ Mexiletine,<br>○ Miconazole,<br>○ Nelfinavir,<br>○ Ritonavir,<br>○ Saquinavir,<br>○ Tacrine,<br>○ Telithromycin,<br>○ Thiabendazole,<br>○ Ticlopidine.<br>○ Verapamil,<br>○ Zileuton |

- Other approved or investigational systemic anticancer treatments, including chemotherapy, hormone therapy and immunotherapy.

- Other investigational drugs.

The use of potent antacids such as proton pump inhibitors (except those listed above) and histamine H2 antagonists is permissible, if medically necessary. However, patients requiring chronic antacid therapy should avoid their use for 2 hours before and for 2 hours after taking axitinib tablets.

The contraindicated medications detailed above may only be used in exceptional circumstances and following written confirmation from SCTRU of the CI's approval.

The current version of the SmPC should be used to assess whether any other concomitant medications are permissible.

Other supportive medicines such as thyroid replacement therapy, anti-emetics and anti-diarrhoeal medicines are permitted in accordance with local practice.

### 4.5. Compliance with Treatment

The study drug must not be used outside the context of the NAXIVA protocol.

Patients must be asked to bring all their trial medication every time they attend the clinic for the purposes of treatment compliance assessment and drug accountability. Every effort should be made to encourage patients to return the unused medication and empty packs. The unused tablets should be collected by the investigator/study nurse and counted to ascertain patient compliance, medication will then be returned to pharmacy for drug accountability prior to destruction according to local practices. Drug accountability and destruction records should be maintained by the local pharmacy.

### 4.6. Drug Supplies and Labelling

**Drug Supply**
Axitinib is supplied free of charge by Pfizer to Tayside Pharmaceuticals as 1mg and 5mg immediate release film-coated tablets and supplied in blister packs. Each 1mg and 5mg carton will contain 56 tablets (four blister sheets of 14 tablets per pack).

Tayside Pharmaceuticals will repack 2 intact blister sheets of 14 tablets each into a new carton along with the patient information leaflet (PIL) for each strength of axitinib. Each carton will then be labelled as per approved label. Release of the re-packaged drug will be carried out by a Qualified Person (QP) at Tayside Pharmaceuticals. IMP will then be dispatched to sites. The schedule of responsibilities for this arrangement is detailed in the contract between Tayside Pharmaceuticals and the sponsor.

SCTRU will arrange for a supply of axitinib to be sent from Tayside Pharmaceuticals to the relevant pharmacy department following centre initiation. Local R&D approval and a study agreement must be in place before any drug can be shipped to sites.

Sites will receive an initial supply of axitinib after completion of site initiation. Each site will receive; 2x 5mg pack as a starting dose and 3x 1mg packs of axitinib to cover any unexpected dose adjustments within the first 2 weeks of the patient schedule.
SCTRU will closely monitor axitinib supply at sites however sites will be expected to notify SCTRU when stock is required as per the NAXIVA Pharmacy Manual. A trial specific order form will be included in the pharmacy pack. Sites must have a minimum of 1x 5mg pack and 3x 1mg packs of axitinib in stock during the recruitment period.

**Labelling**
All study drug supplied by Pfizer will be re-packaged and labelled according to Annex 13 of the 'Good Manufacturing Practice'. The drugs will be re-packaged, labelled, QP released and distributed by Tayside Pharmaceuticals to sites.

### 4.7. Drug Storage Accountability

**Storage**
Axitinib should be stored in the original package in order to protect from moisture and should be stored at controlled room temperature (between 15°C and 30°C). The local pharmacy is responsible for ensuring that the study medication is stored in an appropriate secured area. Study treatment must be kept out of the reach and sight of children.

**Accountability**
The investigator or a delegated individual (e.g. pharmacist) must ensure that the study drug is stored and dispensed in accordance with hospital standard operating procedures and applicable regulatory requirements.

The medication provided for this study is for clinical trial use only as directed. Drug distribution and accountability logs will be provided to the site in a pharmacy pack. It is the investigator's responsibility to establish a system for handling the investigational product to ensure that:

- Deliveries of investigational products from Pfizer via Tayside Pharmaceuticals are correctly received by a responsible person (e.g. pharmacist or suitable pharmacy designee) and are handled and stored correctly and safely.
- Investigational products are dispensed only to study participants, and in accordance with the protocol.
- Participants return any unused investigational product and all empty packs to the investigator.
- A dispensing record (which will include the identification of the participant to whom the investigational product was dispensed, the date of dispensing, the quantity of investigational product dispensed, and the date and quantity of any unused investigational product returned to the pharmacy) is accurately maintained. Any discrepancies must be accounted for on the appropriate form.

In the case that any study drug is damaged, please contact SCTRU for reconciliation and replacement.

At the termination of the study or at the request of the sponsor, all unused drugs will be accounted for and destroyed locally at the study sites. Certificates of delivery and destruction or return must be signed and copies retained in the Investigator Site File.

Accountability records must be completed and any study drug remaining at the end of the trial must be destroyed according to the sites local standard procedures.

# 5. ASSESSMENT OF EFFICACY

## 5.1. Treatment and Examination Schedule

| Activity | Screening (Up to 4 weeks prior to enrolment) | Pre-treatment Day 1, Week 1 | Week 3 | Week 5 | Week 7 | Week 9 | Week 9 (min 36 hours after stopping axitinib) | 6 week post surgery follow up | 12 week post surgery follow up |
|---|---|---|---|---|---|---|---|---|---|
| Confirmation of histological diagnosis of ccRCC via image guided biopsy (5 cores: 1 for diagnosis and 4 cores for research as per appendix 6) | X | | | | | | | | |
| Medical History | X | | | | | | | | |
| Physical examination including ECOG & Karnofsky performance status, body weight, height, temperature, blood pressure[2], heart rate, respiratory rate. | X | X[1] | X | X | X | X | | X | X |
| Haematology, coagulation, glucose, renal and liver function, serum calcium, CRP and LDH. | X | X[1] | X | X | X | X | | X | X |
| Urinalysis for protein | X | X[1] | X | X | X | X | | | |
| Serum Thyroid Function Tests (Free T4 and TSH) | X | X[1] | X | X | X | | | | |
| Pregnancy Test (serum or urine) if applicable | X | | | | | | | | |
| CT Chest, Abdomen and Pelvis for IVC VTT and primary tumour assessment using RECIST 1.1 and Mayo classification | X (all) | | | | | X (M1 only) | | | X (all) |
| CT Chest to check for development of metastasis | | | X (M0 only) | | | X (M0 only) | | | |
| Contrast enhanced MRI abdomen | X | | X | | | X | | | |
| Blood samples and urine sample for biomarker analysis | | X | X | X | X | X | | | X |
| Assessment of compliance with study medication | | | X | X | X | X | | | |
| Dispense axitinib | | X | X | X | X | | | | |
| Dose Escalation (5 to 7 to10mg BID) (depending on toxicities and blood pressure) [3] | | | X | X | X | | | | |
| Stop axitinib (minimum 36 hours, maximum 7 days prior to surgery) | | | | | | X | | | |
| Assessment of concomitant medications | | X | X | X | X | | | | |
| Assessment of symptoms (CTCAE V4) | X | X | X | X | X | X | | X | X |
| Nephrectomy and IVC tumour thrombectomy | | | | | | | X | | |

[1] Laboratory and clinical assessments day 1 week 1 may be omitted if conducted within previous 7 days as part of screening assessment (apart from blood and urine for biomarker analysis).
[2]Blood pressure readings are required at each time point and should be taken by a healthcare professional using an appropriate calibrated machine.
[3]Aggressive increasing of axitinib dose at each to ensure effective dose reached in window period.

## 5.2. Schedule of Assessments

### 5.2.1. Screening Procedures

Screening procedures within 4 weeks prior to study enrolment.

- Clinical assessment
  - Signed informed consent
  - Medical history
  - ECOG & Karnofsky performance status (appendix 1)
  - Body weight
  - Height
  - Blood pressure
  - Heart rate
  - Respiratory rate
  - Temperature
  - Symptom assessment (CTCAE V4)

- Histological assessment
  - Confirmation of diagnosis of ccRCC via image guided biopsy (5 cores should be taken for diagnosis/research as per the 'translational research studies' in appendix 6)

- Laboratory determinations
  - FBC, coagulation, glucose, renal and liver function, serum calcium, C - reactive protein (CRP) and Lactate Dehydrogenase (LDH).
  - Urinalysis
  - Serum thyroid function tests (Free T4 and Thyroid stimulating hormone (TSH)).
  - Pregnancy Test (serum or urine) if applicable (must be performed a maximum of 14 days prior to receiving 1$^{st}$ dose of axitinib)

- Radiological assessment
  - CT thorax, abdomen and pelvis for IVC VTT and primary tumour assessment using RECIST 1.1 criteria and the Mayo classification
  - Diffusion/ contrast enhanced MRI abdomen

### 5.2.2. Study Enrolment

The participant's research nurse and/or doctor will screen the participant to ensure that they meet the trial eligibility criteria, after obtaining participant consent for any additional trial procedures.

Patients will be enrolled centrally with the Scottish Clinical Trials Research Unit (SCTRU). An eligibility and enrolment checklist must be completed prior to enrolment. Enrolment should take place within 72 hours prior to the planned start date of axitinib.

Once consent has been obtained and eligibility confirmed the participant should be enrolled by emailing the NAXIVA team at the Scottish Clinical Trials Research Unit:

**SCTRU enrolment email: NSS.SCTRU@nhs.net**

Referring to the NAXIVA site instructions, the following information will be required at enrolment;

- Name of hospital, consultant and person enrolling the patient
- Confirmation that the patient has given written informed consent for trial participation and the provision of biological samples.
- Confirmation that the patient is eligible for the trial by completion of the eligibility checklist.
- Patients initials, date of birth, sex, NHS/CHI number and date of proven clear cell RCC (metastatic/ non-metastatic).
- Proposed start date of axitinib (must be within 72 hours of enrolment).

The site will inform the participants General Practitioner (GP) of the participants enrolment, if the participant gives consent to do so.

It may be possible for participants to be recruited into other clinical trials, but this should be discussed with the CI via SCTRU before this is considered.

### 5.2.3. Pre-treatment Assessment day 1, week 1 (within 7 days)

- Laboratory determinations*
    - FBC, coagulation, glucose, renal and liver function, serum calcium, CRP and LDH
    - Urinalysis
    - Serum thyroid function tests (Free T4 and TSH)
    - Blood and urine samples for biomarker analysis (NB. patients should be asked to fast for 6 hours prior to urine analysis, and to not take axitinib until after their bloods have been sampled for the biomarker analyses)

- Clinical assessment*
    - ECOG & Karnofsky performance status (appendix 1)
    - Body weight
    - Height
    - Blood pressure
    - Heart rate
    - Respiratory rate
    - Temperature
    - Symptom assessment (CTCAE V4)
    - Assessment for concomitant medications.

*Laboratory and clinical assessments day 1 week 1 may be omitted if conducted within previous 7 days as part of screening assessment (apart from blood and urine for biomarker analysis).

- Radiological assessment
    - CT thorax, abdomen and pelvis for IVC VTT (all patients) and primary tumour assessment using RECIST 1.1 criteria and the Mayo classification (is not required if CT carried out at screening/diagnosis)
      Imaging to be acquired following intravenous contrast medium assuming no contraindications. Thin slice (at least 2mm) imaging will be acquired as an arterial phase in the throax and as a portal venous phase of the abdomen and pelvis.

- Diffusion/ contrast enhanced MRI abdomen (all patients), (is not required if contrast enhanced MRI carried out at screening/diagnosis)
  Thin slice (or volumetric imaging) will be acquired of the kidneys and adjacent IVC to include the tumour thrombus and proximal normal IVC to allow reconstruction of the VTT in axial and coronal planes. Sequences may include:
    T2-weighted imaging
    T1-weighted imaging with and without fat saturation
  Following intravenous contrast injection of a suitable gadolinium-based contrast agent as a dynamic acquisition
    Diffusion weighted imaging
  Non-contrast MR venography of the renal veins and IVC including the tumour thrombus and normal venous lumen adjacent to the thrombus
  The patient may be asked to undergo breathing strategies to enhance imaging (eg. breathhold, valsalva).

- Pharmacy
  - Dispense axitinib

### 5.2.4. Assessment at day 1, week 3 (within 7 days)

- Laboratory determinations
  - FBC, coagulation, glucose, renal and liver function, serum calcium, CRP and LDH
  - Urinalysis
  - Serum thyroid function tests (Free T4 and TSH)
  - Blood and urine sample for biomarker analysis (NB. patients should be asked to fast for 6 hours prior to urine analysis)

- Clinical assessment
  - ECOG & Karnofsky performance status (appendix 1)
  - Body weight
  - Height
  - Blood pressure
  - Heart rate
  - Respiratory rate
  - Temperature
  - Symptom assessment (CTCAE V4)
  - Assessment for concomitant medications
  - Assessment of compliance with study medication
  - Dose evaluation and escalation (where appropriate)

- Radiological assessment
  - CT chest for IVC VTT (M0 patients only) to check for development of metastasis
  - Diffusion/ contrast enhanced MRI abdomen (all patients)

- Pharmacy
  - Dispense axitinib (dose modification as per section 4.2 if applicable)

### 5.2.5. Assessment at day 1, week 5 (within 7 days)

- Laboratory determinations
  - FBC, coagulation, glucose, renal and liver function, serum calcium, CRP and LDH
  - Urinalysis
  - Serum thyroid function tests (Free T4 and TSH)
  - Blood, urine sample for biomarker analysis (NB. patients should be asked to fast for 6 hours prior to urine analysis)

- Clinical assessment
  - ECOG & Karnofsky performance status (appendix 1)
  - Body weight
  - Height
  - Blood pressure
  - Heart rate
  - Respiratory rate
  - Temperature
  - Symptom assessment (CTCAE V4)
  - Assessment for concomitant medications
  - Assessment of compliance with study medication
  - Dose evaluation and escalation (where appropriate)

- Pharmacy
  - Dispense Axitinib (dose modification as per section 4.2 if applicable)

### 5.2.6. Assessment at day 1, week 7 (within 7 days)

- Laboratory determinations
  - FBC, coagulation, glucose, renal and liver function, serum calcium, CRP and LDH
  - Urinalysis
  - Serum thyroid function tests (Free T4 and TSH)
  - Blood and urine sample for biomarker analysis (NB. patients should be asked to fast for 6 hours prior to urine analysis)

- Clinical assessment
  - ECOG & Karnofsky performance status (appendix 1)
  - Body weight
  - Height
  - Blood pressure
  - Heart rate
  - Respiratory rate
  - Temperature
  - Symptom assessment (CTCAE V4)
  - Assessment for concomitant medications
  - Assessment of compliance with study medication
  - Dose evaluation and escalation (where appropriate)

- Pharmacy
  - Dispense axitinib (dose modification as per section 4.2 if applicable)

### 5.2.7. Assessment at day 1, week 9 (within 7 days)

- Laboratory determinations
  - FBC, coagulation, glucose, renal and liver function, serum calcium, CRP and LDH
  - Urinalysis
  - Blood and urine sample for biomarker analysis (NB. patients should be asked to fast for 6 hours prior to urine analysis)

- Clinical assessment
  - ECOG & Karnofsky performance status
  - Body weight
  - Height
  - Blood pressure
  - Heart rate
  - Respiratory rate
  - Temperature
  - Symptom assessment (CTCAE V4)
  - Assessment of compliance with study medication

- Radiological assessment
  - CT thorax, abdomen and pelvis (M1 patients) for IVC VTT and primary tumour assessment using RECIST 1.1 criteria and the Mayo classification

  - CT chest (M0 patients) for IVC VTT to check for development of chest metastasis.

  - Diffusion/ contrast enhanced MRI abdomen (all patients)

The patient should stop axitinib a minimum of 36 hours and a maximum 7 days prior to surgery; no further study drug should be dispensed.

### 5.2.8. Surgery day 2-7, week 9 (+/- 7 days)

- Surgery
  - Nephrectomy and IVC tumour thrombectomy

The surgeon should complete the surgery CRF on the day of surgery.

### 5.2.9. Follow up assessment- 6 weeks post surgery (within 7 days)

- Laboratory determinations
  - FBC, coagulation, glucose, renal and liver function, serum calcium, CRP and LDH

- Clinical assessment
  - ECOG & Karnofsky performance status (appendix 1)
  - Body weight
  - Height
  - Blood pressure
  - Heart rate

- Respiratory rate
- Temperature
- Symptom assessment (CTCAE V4)

### 5.2.10. Follow up assessment- 12 weeks post surgery (within 7 days)

- Laboratory determinations
  - FBC, coagulation, glucose, renal and liver function, serum calcium, CRP and LDH
  - Blood and urine sample for biomarker analysis (NB. patients should be asked to fast for 6 hours prior to urine analysis)

- Clinical assessment
  - ECOG & Karnofsky performance status (appendix 1)
  - Body weight
  - Height
  - Blood pressure
  - Heart rate
  - Respiratory rate
  - Temperature
  - Symptom assessment (CTCAE V4)

- Radiological assessment
  - CT thorax, abdomen and pelvis (all patients) for restaging.

### 5.2.11. Imaging

- All imaging data will be collated centrally.
- CT and RECIST assessment will be undertaken locally.
- MRI assessment will be undertaken at a single site to ensure consistency.

<u>Radiological endpoints</u>

Primary
- The VTT position in relation to the IVC for the Mayo classification will be performed using a combination of both MRI and CT.

Secondary
- RECIST 1.1 criteria will be used for CT evaluation

Exploratory
- The following exploratory endpoints may also be evaluated on MRI
  - VTT volume will be calculated using the thin slice MRI images reconstructed where appropriate. A small group of readers will undertake this and where possible automated segmentation will be used for consistency.
  - Change in the maximum VTT length using coronal MRI reconstructions
  - Apparent Diffusion Coefficient (DWI) changes over time of both the whole tumour and VTT.
  - Textural features of the whole tumour and VTT on MRI as a measure of heterogeneity, as well as changes in heterogeneity during treatment. This will be measured using semi-automated proprietary software where possible.

### 5.3. Withdrawal of Subjects

Patients *discontinue* from the study for reasons such as safety, non-compliance or withdrawal of consent, etc. These patients will continue to be followed up for efficacy and safety as per protocol, unless they are lost to follow up, deceased or withdrew consent to the study. The bio-samples collected during the study will continue to be used for translational research.

Patients may *withdraw* from the study at any point; no further data collection will take place, and will be censored at the point of withdrawal this should be indicated on the case report form (CRF) as per completion guidelines.

Patients who consent to the trial but do not start trial treatment will be withdrawn from the trial. These patients will not be followed up as part of the study and will be expedited to surgery as per standard practice.
For any patient that did not start study treatment, a further patient would be enrolled to the trial to maintain the target number of patients for analysis.

## 6. Translational Research

Tissue, blood and urine will be collected at baseline (all sample types), throughout the 8 week treatment period (blood and urine) and at surgery (all sample types). A minimum requirement will be access to a formalin-fixed, paraffin-embedded tumour block (excess to diagnostic requirements) from the patient biopsy and nephrectomy. Where the institutional infrastructure exists the following samples will be collected: fresh frozen tissue (biopsy and nephrectomy), fresh tissue (biopsy and nephrectomy) plasma, buffy coat, urine, and peripheral blood mononuclear cells (PBMCs). The analysis streams will be as follows, with the aim of identifying molecular correlates to the clinical outcomes of this study.

### 6.1. Circulating tumour DNA (ctDNA)

In view intratumoural heterogeneity, the use of tumour tissue for the development of prognostic and predictive biomarkers in ccRCC is challenging (20). Circulating tumour DNA (ctDNA) has been established to represent the tumour biology and may be superior to tissue as heterogeneity will be negated (21). Obtaining ctDNA from blood from patients with VTT is the ideal platform on which to validate ctDNA in RCC because of the high likelihood of DNA shedding from the tumour directly into the circulation. Furthermore, the change in ctDNA profiles from baseline to treatment phase to post-treatment phase may be informative regarding markers of tumour response.

### 6.2. Metabolomics

It is well established that there is a strong metabolic basis to ccRCC. The presence of the Warburg effect has revealed several novel targets for treatment or tumour surveillance (22). Metabolites will be assayed in urine, blood and tissue across the various time points of the NAXIVA study to assess the use of these molecules in patients with VTT and with axitinib therapy.

### 6.3. Predictive biomarker studies

There has been previous identification of a prognostic biomarker signature for response to sunitinib. The same biomarker profile will be validated for axitinib using the matched biopsy and nephrectomy tumour tissue samples obtained in this study.

### 6.4. Comparative study of IVC VTT in untreated and treated patients

Dovetailing in with ongoing studies looking at the invasive front of the IVC VTT in TKI naïve patients, the VTT samples from NAXIVA will be assessed using the same biomarker pipeline specifically searching for evidence in alterations in EMT or invasion profiles. The hypothesis being that treatment with axitinib abrogates some of the invasive features of the VTT.

### 6.5. Imaging

There is little published data on the best imaging method for assessing the IVC VTT. USS, CT and MRI are all currently used in different centres. Surgical outcome studies have shown that patients with solid VTT will have a superior oncological outcome compared to friable VTT, yet there are no radiological data on how to make this assessment. A non-invasive imaging biomarker of VTT composition would help to guide surgery in patients with borderline fitness. Textural analysis of the serial CT and MRI scans undertaken as part of NAXIVA will be performed and compared to the surgical outcome to assess if VTT composition and the effect of drug on VTT can be determined on imaging. Renal vein and IVC VTT volumes will be measured using MR venography techniques developed in Cambridge and % changes in post-contrast enhancement within the thrombus will be assessed from dynamic contrast-enhanced MRI (DCE-MRI) data. Exploratory analyses will include: changes in total tumour volume in the renal vein and IVC (CT and MRI); changes in the degree of enhancement after intravenous contrast administration (MRI); changes in imaging heterogeneity (texture) of the renal and IVC VTT (CT and MRI); differentiation of solid from friable IVC VTT using MR venography and physiological manoeuvres (Valsalva).

### 6.6. Immune Analysis

It is known that RCC is a typical immunogenic tumour with immune mechanisms known to play an important role in its natural history. Recent studies have also shown that TKI treatment can influence the immune system and, importantly, changes in the immune response may affect response and survival. The immune aim to identify immune- and cytokine-based signatures in PBMCs, plasma and urine which predict response to axitinib treatment in RCC patients. These studies will also provide important information about the effect of axitinib on the immune system in RCC, and vice versa, including providing comprehensive analysis of how peripheral immune cell populations and cytokines vary during treatment with axitinib, and whether they correlate with other markers of response and resistance in RCC patients. Importantly, it will also provide essential immunological information to inform biologically driven, rational, scheduling of future studies examining combinations of axitinib and immunotherapy.

## 7. PHARMACOVIGILANCE

### 7.1. Definitions

**Adverse Event (AE):** An adverse event (AE) is any untoward medical occurrence in a patient which does not necessarily have a causal relationship with the study treatments or procedures. An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with a treatment or procedure, whether or not considered related.

**Adverse Reaction (AR):** All noxious and unintended responses related to a study treatment or procedure should be considered adverse drug reactions.

**Serious Adverse Event (SAE):** Any untoward medical occurrence in a patient that

a) Results in death
b) Is life-threatening
c) Requires hospitalisation or prolongation of existing hospitalisation
d) Results in persistent or significant disability or incapacity
e) Consists of a congenital anomaly or birth defect
f) Is otherwise considered to be medically significant by the investigator

The term "life-threatening" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

Hospitalisations planned prior to enrolment in the trial or for social reasons should not normally be considered as SAEs unless the hospitalisation has to be prolonged. Treatment in an emergency room of less than 24 hours or on an out-patient basis that does not meet any other serious criteria should not be considered as an SAE.

Hospitalisation for nephrectomy is not considered a SAE where this goes ahead as planned within the trial. Prolonged hospitalisation, however, should be reported as SAE.

**Suspected Unexpected Serious Adverse Reaction (SUSAR):** A suspected unexpected serious adverse reaction (SUSAR) is an adverse reaction that is classified as serious and it is suspected that it is caused by a study treatment or procedure. The nature, severity or outcome of this adverse reaction also must not be consistent with the current version of the SmPC for the treatment or procedure.

### 7.2. Recording of Adverse Events

All related Adverse Reactions will be recorded in the Case Report Form. Any Adverse Reaction considered unrelated will not be recorded.
All adverse reactions that occur after the signing of written informed consent and within 30 days after the final study treatment will be recorded on the appropriate CRF page. The exception to this would be any event occurring after signing the informed consent and prior to commencing study treatment that is considered unrelated to trial procedures. In addition any events occurring more than 30 days after final study treatment that are deemed to be related to the study drug should be notified to SCTRU as detailed in section 7.4.

Any medical conditions or diseases present prior to signing of informed consent should only be considered an adverse event if there is a worsening of the condition.

Please refer to Appendix 2 for details on the Causality.

### 7.3. Recording and Reporting of Serious Adverse Events

| **Contact Details for Reporting SAEs** | |
|---|---|
| **SCTRU Fax:** | +44 131 275 7512 (preferred method) |
| **SCTRU Telephone:** | +44 131 275 7276 (Mon – Fri 9am-4pm) |
| **Or:** | +44 131 316 4278 (Mon – Fri 9am-4pm) |

All serious adverse events that occur after the signing of written informed consent and within 30 days after the final study treatment will be recorded on the SAE report form. The exception to this would be any event occurring after signing the informed consent and prior to commencing study treatment that is considered unrelated to trial procedures. In addition any events occurring more than 30 days after final study treatment that are deemed to be related to the study drug should be notified to SCTRU as above.

The SAE report form must be signed by the Principal Investigator (PI) of the centre involved and faxed to SCTRU within 24 hours of first becoming aware of the event. All initial SAE reports should contain the following minimum information:

- Reporter information
- At least one subject identifier (trial number/patient initials)
- Event term
- Assessment of relatedness
- Serious criteria

A fax or email receipt will be sent to the relevant centre by SCTRU to acknowledge receipt of the SAE report form, and SCTRU will notify the CI.

All SAEs will be forwarded to the CI by SCTRU for assessment of expectedness against the current SMPC. Any SAE that is deemed to be both related and unexpected (i.e. a SUSAR) will be notified to the appropriate Competent Authorities and Research Ethics Committees within 7 days of becoming aware of the event for fatal or life threatening events and 15 days for all other serious events.
SUSARs should be reported to the Research Ethics Committee (REC) accompanied by the 'safety reports to the REC covering form'. The coordinator of the REC should acknowledge receipt of the safety report within 30 days by signing and returning a copy of the covering form.
SCTRU will then notify the CI and the PI's at all of the participating centres of the occurrence of all SUSARs.

Hospitalisations planned prior to enrolment in the trial or for social reasons should not normally be considered as SAEs unless the hospitalisation has to be prolonged. Treatment in an emergency room of less than 24 hours or on an out-patient basis that does not meet any other serious criteria should not be considered as an SAE.

### 7.4. Developmental Safety Update Report

An Annual Safety Report will be submitted to the appropriate Competent Authorities and Ethics Committees, once a year for the duration of the trial. The time frame for the report starts with the date of first authorisation by a competent authority in an EU member state and the report should be submitted within 60 days of the anniversary of first authorisation.

### 7.5. Pregnancies

Any pregnancy in a trial participant or their partner that occurs during study participation should be reported to SCTRU within 24 hours of becoming aware of its occurrence, using the contact details in Section 7.3. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary abortion, details of birth and presence or absence of any birth defects, congenital abnormalities or maternal or newborn complications. Any birth defects or congenital abnormalities must be reported as SAEs.

# 8. DATA MANAGEMENT

All data will be handled, computerised and stored in accordance with the Data Protection Act 1998 and NHS National Services Scotland Confidentiality Guidelines.

## 8.1. Data Collection

Data generated will be collected by SCTRU. SCTRU will be responsible for checking the data, and validating it. The data collected will include:

- initial clinical details at registration
- drug administration (CTIMPs)
- concomitant medications
- adverse events
- survival/ recurrence details
- surgical techniques
- tumour measurements
- vena cava thrombus measurement
- Dose escalation details

## 8.2. Record Keeping and Archiving

SCTRU will store study documentation until the end of patient follow up. The documentation will then be archived according to current legislative requirements.

# 9. STATISTICS

## 9.1. Sample Size

The aim is to recruit 20 patients over a 24 month period. A Simon two stage minimax design to distinguish a <5% from a >25% improvement in the Mayo classification requires 20 patients (90% power, 10% 1-sided). Thirteen patients would be recruited at first stage; if at least 1 patient had an improvement in the Mayo classification a further 7 patients would be recruited (to the final total of 20 patients).

## 9.2. Analysis Plan

The main analysis will be conducted on the “as-treated” population. All patients who receive study drug will be included in the interim and final analysis. Any patient who withdraws from the study prior to receiving drug will not be included in the analysis. At least 3 patients showing a reduction in Mayo Classification would produce a positive trial.

**Interim Analysis**
An interim analysis will be performed after thirteen patients have been recruited. If no patients have show an improvement in their Mayo classification the trial will be stopped for futility. If at any point in the study 3 patients have a progression of their thrombus (increase in Mayo classification) but remain surgically resectable the trial will be stopped. (Probability of seeing 3 or more progressions if true progression rate <5% is <7.5%). There is no formal stopping rule for efficacy.

The primary analysis will be made on the “treated” population, with patients being included in the analysis only if they have received at least one dose of the drug. In order to assess the impact of any biases an analysis of the “intention to treat” population will also be carried out.

This will include all patients who are registered as eligible for the trial, regardless of whether or not the subsequently received study medication.

**Final Analysis**
The final analysis will be performed once the end of study has been declared and will include summaries of the % of evaluable patient with improvement in the Mayo classification; % change in surgical approach; the response rates as determined by RECIST criteria V1.1 and an evaluation of morbidity utilising the Clavien-Dindo classification system.

The dose of axitinib delivered will be summarised with the median and range of total dose being presented. Adverse events will be classified using CTCAE V4 and the worst grade for each toxicity will be summarised.

### 9.3. End of Study

This study will end when the below criteria are deemed complete by the sponsor; Last patient last visit (12 week post surgery follow up) complete and the database is clean and frozen for analysis.

## 10. ACCESS TO SOURCE DATA/ DOCUMENTS

The investigator, by accepting to participate to this protocol, agrees to co-operate fully with any quality assurance visit undertaken by third parties, including representatives from the Sponsor, SCTRU, national and/or foreign regulatory authorities or company supplying the product under investigation, as well as to allow direct access to documentation pertaining to the clinical trial (including CRFs, source documents, hospital patient charts and other study files) to these authorised individuals.

## 11. QUALITY CONTROL AND QUALITY ASSURANCE

Quality control will be maintained through adherence to appendix 7, Good Clinical Practice (GCP) and the coordinating centre's SOPs. The coordinating centre will monitor receipt of CRFs and evaluate incoming CRFs for compliance with the protocol, inconsistencies and missing data.

### 11.1. Monitoring Visits

We have allowed for site visits in the UK to enable monitoring by SCTRU to check patient consent forms, confirm compliance with the protocol and complete source data verification (SDV) on the patient data as defined in the Data Monitoring Plan. Higher levels of monitoring will be performed, if requested, by the Data Monitoring Committee, or if the investigators, the Trial Management group or Trial Steering Committee identify particular safety issues.

### 11.2. Data Monitoring and Ethics Committee

An independent Data Monitoring Committee (DMC) will be established and will meet 6 monthly in the first instance and annually thereafter (and at any other time at the committee's discretion). There will be an extra meeting of the committee after 13 patients have been recruited. None of the committee members will be involved in the trial. The committee will receive regular reports from SCTRU. It will submit its comments and recommendations to the Trial Steering Committee and Trial Management Group (TMG).

### 11.3. Trial Steering Committee

A trial steering committee will be established to provide overall supervision of the trial, in particular; trial progress, adherence to protocol, patient safety, and consideration of new information. The committee will meet 3 monthly in the first instance and then 6 monthly thereafter. The committee will then meet 3 monthly during close out/ final analysis.

## 12. ETHICAL CONSIDERATIONS

Ethical approval by the East of England – Cambridge Committee will be required before the trial can be started.
The trial will be carried out according to GCP guidelines as defined by paragraph 28 and Schedule 1 Part 2 of the Medicines for Human Use (Clinical Trials) Regulations, 2004, and the Clinical Trials Directive (2001/20/EC) elsewhere in the European Union and follow the principles of research governance.

The use and storage of human tissue will be carried out in accordance with The Human Tissue Act (2004) and The Human Tissue (Scotland) Act (2006). Human Tissues is defined as any material which has come from a human body that consists of, or includes human cells and includes blood and other bodily fluids.

### 12.1. Patient Confidentiality

The patient's full name, date of birth, hospital number and NHS number (Community Health Index and/or hospital number in Scotland) will be collected to enable tracing through national records. The personal data recorded on all records will be regarded as confidential, and to preserve each patient's anonymity, only their initials and date of birth will be recorded on CRFs. The patients will be identified within the CRFs by the use of a unique trial number allocated to them upon entry into the study.
The PI (or delegate) at each site must keep a log of patients' trial numbers, names, addresses and hospital numbers. The PI must ensure that patient confidentiality is maintained and that all trial documents (e.g. consent forms) are maintained in strict confidence.

SCTRU will maintain the confidentiality of all patient data and will not reproduce or disclose any information by which patients could be identified. Patients will only be referred to by Trial Number, Initials and Date of Birth in any essential trial related correspondence, including Case Report Forms and Serious Adverse Event Reports.

All patient identifiable data will be handled, computerised and stored in accordance with the Data Protection Act 1998 and NHS National Services Scotland Confidentiality Guidelines.

### 12.2. Informed Consent

All patients will be informed of the aims of the study, the possible adverse events, the procedures and possible hazards to which they will be exposed, and the mechanism of treatment allocation. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician. It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever they want. This will not prejudice the patient's subsequent care.

Documented informed consent must be obtained for all patients included in the study before they are enrolled. This must be done in accordance with the national and local regulatory requirements and must conform to guidelines on Good Clinical Practice. That is, "the written informed consent form should be signed and personally dated by the patient or by the patient's legally acceptable representative".

Copies of the patient information sheets and consent forms are provided in appendices 4 & 5.

All Patient Information Sheets & Informed Consent Forms will be version controlled and dated and this information will always be stated in any communication with ethics committees.

## 13. RESEARCH GOVERNANCE

**Sponsor (CSA)** – The sponsor will have overall responsibility for the design, co-ordination and management of the study. These include:

- Trial authorisation including responsibility for the protocol and obtaining approvals
- Ensuring that the trial is conducted according to GCP guidelines (22,23)
- Assessment of SAEs and providing a prompt response as to whether the SAE is a SUSAR.

**Clinical Trials Unit** – The sponsor has delegated the responsibility for overall project management, data management and monitoring to Scottish Clinical Trials Research Unit, NHS National Services Scotland
Responsibilities include:

a. Assistance with completion of the IRAS form and REC communication
b. Production of trial specific documentation (i.e. CRFs)
c. Facilitating set up of trial centres
d. Data management
e. Monitoring
f. Pharmacovigilance – Reporting of SARs / SUSARs

**Statistical Analysis** – A Principal Information Analyst, based at SCTRU, Edinburgh will undertake the final analysis arising for this study.

**Local Project Teams** – These will consist of Surgeons and/or Oncologists (responsible for introducing the patient to the study and ensuring eligibility and consent), Research Nurse (responsible for patient recruitment, obtaining consent and co-ordination of all aspects of data collection), Pathologists (responsible for tissue sample analysis), Radiologists and Radiographers (responsible for completing MRI and CT scans to protocol). Centres are specifically responsible for conducting the trial in accordance with the protocol, Standard Operating Procedures (SOPs), the trial agreement and Good Clinical Practice.

**Trial Steering Committee and Data Monitoring and Ethics Committee** – The Common Services Agency (CSA) will act as study sponsor. Central study co-ordination, data collection, monitoring and organisation of the data for the statistical analyses will be undertaken by Scottish Clinical Trials Research Unit, NHS National Services Scotland, which has processes in place to ensure that the study will not open to recruitment until appropriate

approvals and authorisations have been obtained from the independent research ethics committee, and NHS Research and Development departments (R&D). The Trial Steering Committee (TSC), including members of the research team and an radiologist, oncologist, statistician, and lay members, will be responsible for the progress and conduct of the study and convene annually. A Trial Management Group will meet quarterly. A Data Monitoring and Ethics Committee (DMC) will convene 6 monthly, to review all data including adverse events and review the stopping policy for the trial, if necessary. Specific issues that will be looked at include: tolerability of initial dose, dose reductions, toxicities (including interaction of the mild increased bleeding risk), imaging & review schedules.

**Laboratories** – Accredited local laboratories shall be used for all on-study tests. Samples from the translational research will be transferred to a central laboratory based at the University of Cambridge. The details of the sampling, storage and shipping protocols are covered in appendix 6 and the NAXIVA laboratory manual in accordance with all prevailing regulatory requirements.

## 14. FINANCING AND INSURANCE

This study is funded by Pfizer Limited and endorsed by Cancer Research UK (CRUK). Indemnity for participating hospitals is provided by the usual NHS indemnity arrangements.

## 15. PUBLICATION POLICY

All presentations and publications relating to the trial must be authorized by the Trial Management Group. The main trial results will be published in the name of the trial in a peer-reviewed journal, on behalf of all collaborators. The manuscript will be prepared by Trial Management Group, representatives from SCTRU, NHS National Services Scotland and high accruing clinicians. The trials offices and all participating Centers and clinicians will be acknowledged in this publication. Any data that might detrimentally affect the progress of the trial will not be released prior to the end of the trial. No investigator may present or attempt to publish data concerning their patients, which is directly relevant to the questions posed in the trial, until the main results have been published.

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**Appendix 1 – ECOG and Karnofsky Performance Scores**

| Karnofsky Status | Karnofsky Score | ECOG Grade | ECOG Status |
|---|---|---|---|
| Normal, no complaints. | 100 | 0 | Fully active, able to carry on all pre-disease performance without restriction. |
| Able to carry on normal activities. Minor signs or symptoms of disease. | 90 | 1 | Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work. |
| Normal activity with effort. | 80 | 1 | Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work. |
| Care for self. Unable to carry on normal activity or to do active work. | 70 | 2 | Ambulatory and capable of all self care but unable to carry out any work activities. Up and about more than 50% of waking hours. |
| Requires occasional assistance, but able to care for most of his needs. | 60 | 2 | Ambulatory and capable of all self care but unable to carry out any work activities. Up and about more than 50% of waking hours. |
| Requires considerable assistance and frequent medical care. | 50 | 3 | Capable of only limited self care, confined to bed or chair more than 50% of waking hours. |
| Disabled. Requires special care and assistance. | 40 | 3 | Capable of only limited self care, confined to bed or chair more than 50% of waking hours. |
| Severely disabled. Hospitalisation indicated though death nonimminent. | 30 | 4 | Completely disabled. Cannot carry on any self care. Totally confined to bed or chair. |
| Very sick. Hospitalisation necessary. Active supportive treatment necessary. | 20 | 4 | Completely disabled. Cannot carry on any self care. Totally confined to bed or chair. |
| Moribund. | 10 | 4 | Completely disabled. Cannot carry on any self care. Totally confined to bed or chair. |
| Dead. | 0 | 5 | Dead. |

**Appendix 2 – Causality**

The assignment of causality should be made by the investigator responsible for the care of the patient, using the definitions in the table below.
If any doubt about the causality exists the investigator should inform the Trials Centre who will notify the Chief Investigator.
In the case of discrepant views on causality between the investigator and others, the case will be discussed by all parties. In the event that no agreement is made, the MHRA will be informed of both points of view.

Description of causality of adverse events

| | | |
|---|---|---|
| Possibly Related: No | Unrelated | There is no evidence of any causal relationship. |
| | Unlikely | There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatment). |
| Possibly Related: Yes | Possible | There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments). |
| | Probable | There is evidence to suggest a causal relationship and the influence of other factors is unlikely. |
| | Definitely | There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out. |

**Appendix 3a – Investigator Statement (SCTRU Copy)**

**NAXIVA**

**PHASE II NEOADJUVANT STUDY OF AXITINIB FOR REDUCING EXTENT OF VENOUS TUMOUR THROMBUS IN CLEAR CELL RENAL CELL CANCER WITH VENOUS INVASION (NAXIVA)**

## Principal Investigator Declaration

I acknowledge receipt of version 1.2 dated 22nd September 2017 of the NAXIVA trial protocol (REC approved17th October 2017) and I agree to perform this trial in accordance with this version of the protocol and Good Clinical Practice.

I understand that the safety of the patient is my first concern

Print Name: ----------

Hospital: ----------

Signed: ----------

Date: ----------

Please return this copy to: NAXIVA Trial Coordinator
Scottish Clinical Trials Research Unit,
Gyle Square,
1 South Gyle Crescent,
Edinburgh,
EH12 9EB

**Appendix 3b – Investigator Statement (Investigator Copy)**

**NAXIVA**

**PHASE II NEOADJUVANT STUDY OF AXITINIB FOR REDUCING EXTENT OF VENOUS TUMOUR THROMBUS IN CLEAR CELL RENAL CELL CANCER WITH VENOUS INVASION (NAXIVA)**

## Principal Investigator Declaration

I acknowledge receipt of version 1.2 dated 22nd September 2017 of the NAXIVA trial protocol (REC approved 17th October 2017) I agree to perform this trial in accordance with this version of the protocol and Good Clinical Practice.

I understand that the safety of the patient is my first concern

Print Name: ----------

Hospital: ----------

Signed: ----------

Date: ----------

Please retain this copy and file in Investigator Site File.

**Appendix 4 – Main Study Patient Information Sheet and Informed Consent**

*[To be printed on hopsital headed paper]*

**PATIENT INFORMATION SHEET- Main Study**

**'NAXIVA'**

**Phase II neoadjuvant study of axitinib for reducing extent of venous tumour thrombus in clear cell renal cell cancer with venous invasion**

EudraCT No: 2017-000619-17
ISRCTN96273644
IRAS ID: 223309

PIS Version: 1.2
Version Date: 22nd September 2017

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**Invitation Paragraph**
You are being invited to take part in a research study. Before you decide if you want to take part it is important for you to understand why the research is being done, how your information will be used, what the study will involve and the possible benefits, risks and discomforts. Please take time to read the following information carefully and discuss it with your relatives, friends or GP if you wish.

Please ask if there is anything that is not clear or if you would like further information.

**Main Components of Study**

**1. What is the purpose of the study?**

We want to see if combining standard surgical treatment with a type of drug called a tyrosine kinase inhibitor can stop the growth of the tumour in patients with the type of cancer that you have. Tyrosine kinase inhibitors are a type of drug that act by blocking certain proteins called tyrosine kinases. These proteins tell the cancer cells to grow. Previous studies have suggested that tyrosine kinase inhibitors may slow or stop the growth and in some cases may reduce the size of the tumour.

In the NAXIVA study, axitinib is the tyrosine kinase inhibitor, which is being tested. The drug is already used after surgery for some patients who have metastatic disease. We want to see if giving the drug before surgery (neoadjuvant) would be effective in preventing cancer cells from growing or causing them to shrink. If this happens it may mean that patients need less extensive surgery.

**2. Why have I been invited to take part?**

You have been invited to take part in this study because you have kidney cancer that may be suitable for treatment with a type of drug called tyrosine kinase inhibitor before your surgery. Approximately 20 other kidney cancer patients like you will be asked to take part in the study, in up to 7 hospitals across the UK.

## 3. Do I have to take part?

No, it is up to you to decide whether or not to take part. If you do choose to take part, you will be asked to sign an informed consent form and you will be given a copy to keep together with this information sheet. For your safety your GP will be informed of your participation in this study.

If you do not wish to take part in the study, you do not have to give a reason. You will not be disadvantaged in any way, and it will not affect the standard of care you receive. This also applies if you initially decide to take part and then change your mind at a later date. Your doctor will discuss your treatment options at that time: you do not need to take part in the study to be offered the standard treatment, which is surgery within 4-6 weeks without a tyrosine kinase inhibitor.

## 4. What will happen to me if I decide to take part?

Before you are entered onto the NAXIVA study you will have a biopsy taken of your kidney cancer along with a series of scans, tests and examinations to confirm that you are eligible to take part in the study. This will be done approximately 1 to 4 weeks before you start taking any study medication.

When it's confirmed that you are eligible to take part in the trial you will be seen and examined in clinic before being given your first prescription of axitinib tablets to take at home twice a day. You will be prescribed axitinib at each clinic visit before surgery. After this, you will be seen and examined in clinic weeks 3, 5, 7 and 9 prior to surgery. You will then be followed up at clinic 6 and 12 weeks after surgery.

At each visit you will have routine blood samples of approximately 40ml (3 tablespoons) taken. You will also have your blood pressure checked and be asked to provide a urine sample for dipstick testing.
At each visit, you will be asked about your health and any other medications you are taking. You will also be asked about any side effects or problems you may be having with the study medication.

At the beginning of the study CT and MRI scans will be performed. Following this, you will be assessed to see how the kidney cancer is responding to the study drug. This will be done by subsequent CT/MRI scans at weeks 3 & 9 prior to surgery. If your cancer has not spread to other parts of the body (non-metastatic or M0), you will have a CT scan in week 3. If your cancer has already spread to other parts of your body (metastatic or M1) you will not need this scan. These scans will give your study doctor clearer pictures of the inside of your body. It is very likely that you will have had this type of scan already.

You will stop the axitinib a minimum of 36 hours and a maximum of 7 days prior to surgery. Surgery will be performed at week 9.

After you have completed treatment and surgery, you will be asked to return to clinic for a follow up visit, 6 & 12 weeks after surgery. This is so that your doctor can continue to check on your progress. At the follow up visit you will be asked about your health and about any new treatments you may be receiving. At your final study visit (12 weeks post surgery) you will have a further blood and urine test.

At each visit, you will discuss your progress with your doctor alongside any scan results.

By consenting to NAXIVA you will be expected to attend 2 additional clinic visits compared with standard care; this is so the research staff can monitor your progress on the trial closely. Please find an overview of the trial visits in table 1.

Table 1-Trial Visit Schedule

| | Screening | Visit 1 | Visit 2 | Visit 3 | Visit 4 | Visit 5 | Visit 6 | Visit 7 | Visit 8 |
|---|---|---|---|---|---|---|---|---|---|
| | 1-4 weeks prior to study enrolment | Week 1 | Week 3 | Week 5 | Week 7 | Week 9 | Week 9 | 6 weeks post surgery | 12 weeks post surgery |
| Confirm eligibility | X | | | | | | | | |
| Examined in clinic | X | X | X | X | X | X | | X | X |
| Blood & Urine samples | | X | X | X | X | X | | | X |
| MRI scan | X | | X | | | X | | | |
| CT scan | X | | X (M0 only) | | | X | | | X |
| Axitinib Prescription | | X | X | X | X | | | | |
| Stop axitinib (min 36 hours) prior to surgery | | | | | | X | | | |
| Surgery | | | | | | | X | | |

## 5. What will I be asked to do?

You will need to continue taking the daily tablets that are prescribed for you and to attend clinic visits at the time intervals as already mentioned.

At each visit you will also be asked to return any medicine boxes you have (even if they are empty) and unused tablets to the hospital.

Prior to each visit you will be asked to fast for 6 hours. This is to make sure that your tests can be interpreted correctly by your doctor.

At each visit you will be asked to provide a urine sample for dipstick analysis.

## 6. How long will I be on treatment for?

Your course of axitinib will continue for 8 weeks until surgery. You will stop study treatment at a minimum of 36 hours and a maximum of 7 days prior to surgery.

## 7. What other medicines or treatments could I have instead?

Your doctor will discuss any alternative available treatments with you depending on what is best for you. One option will likely be the current established treatment of urgent surgical removal.

## 8. Can I continue taking axitinib after the trial has ended?

You will take your final axitinib before your surgery. After your surgery, your local hospital team will decide on the appropriate follow up treatment. Only patients whose cancer has already spread to other places may be offered another type of Tyrosine Kinase Inhibitor.

## 9. What are the possible disadvantages and risks of taking part?

One disadvantage is that you may be required to attend clinic more often than people who are not in the study. This is because the research staff may want to see you more often to check on your progress.

You may also have to have additional blood and urine tests and also scans that you would not have if you were not on the study. Because you are in a clinical trial, we want all patients to have these tests/scans at the same time points.

By taking part in the NAXIVA trial you will have an additional MRI scan and if your cancer has not spread you will also have an additional CT scan compared to if you were not taking part in NAXIVA. For both MRI and CT scans, you will receive an additional injection of a contrast agent. There is a small risk of side-effects from these contrast agents such as experiencing flu-like symptoms, a rash or difficulty breathing but, again, this risk is unlikely to be significant. These side effects occur in 2-3 people per 100 and the risk of experiencing a severe reaction occurs in 1 in 1000 people or fewer.

Having an additional CT scan carries a risk of exposure to radiation; the lifetime risk of developing cancer from this radiation is approximately 1 in 1400. This small risk should be balanced against the normal lifetime risk of cancer which is approximately 1 in 3.
There is no additional risk of exposure to radiation by having an additional MRI scan.

Another disadvantage is of course that you may experience side effects from the medicines you will be taking (see section 11).

## 10. What are the possible benefits of taking part?

If you do wish to participate in this study we cannot be sure that there will be a direct benefit for you. Surgery for removing kidney cancer that has spread into the renal vein and/or inferior vena cava is major surgery with a risk of complications.

Our hope is that axitinib will stop your cancer from growing which will improve surgical results by enabling your surgery to be a less extensive operation. This may mean a smaller incision and a less invasive operation on the inside of your abdomen. If this is the case, we hope for fewer complications (such as less blood loss and less chance of requiring a blood transfusion), a shorter hospital stay and faster recovery time at home.

We also hope this research will teach us more about the type of cancer that you have and how it can be treated. This may enable us to improve the standard of treatment to help other patients with cancer in the future.

## 11. What are the possible side effects of the medicines

The study medication may cause some side effects. No one can predict whether you will have some, all or none of these, or how severe they may be. It is important that you tell your study doctor or research nurse about any problems you have at each hospital visit. You can

telephone either of them between visits if you are concerned and you will find their telephone numbers printed at the end of this information sheet.

Very Common side effects of axitinib
( More than 10 in 100 people have one or more of these side effects)

- Flu-like symptoms (Headaches, Fatigue, Joint and Muscle pain)
- Loss of appetite, weight loss
- Nausea, Vomiting and Diarrhoea
- A drop in the level of thyroid hormones (hypothyroidism).
- A sore mouth, tongue or throat
- Raised blood pressure (hypertension)
- An increased risk of bleeding, including nosebleeds and bleeding gums
- Shortness of breath and a cough
- Tummy (abdominal) pain
- Constipation
- Indigestion
- Taste changes or loss of taste
- Proteinuria (protein in the urine)
- Hand-foot syndrome (a skin reaction on your hands/feet which may cause pain, swelling, redness, peeling skin, blisters, tingling or numbness of the hands/feet).

Common side effects of axitinib
(Between 1 and 10 in every 100 people have one or more of these effects)

- Tiredness and breathlessness due to a drop in red blood cells (anaemia)
- An overactive thyroid gland (Hyperthyroidism)
- Dehydration
- Skin changes (rash or red, dry, itchy skin)
- Hair Thinning (Alopecia)
- Dizziness
- Ringing in the ears (tinnitus)
- Low levels of platelets in the blood – you may have nosebleeds, or bleeding gums after brushing your teeth, or you may have lots of tiny red spots or bruises on your arms or legs (known as petechiae).
- High levels of platelets leading to blood clots. If it happens to you, you will have treatment to thin your blood, dissolve any clots, and stop any more developing.
- Mild effects on the liver and kidneys, which are unlikely to cause any symptoms and will usually go back to normal after the treatment ends. You will have regular blood tests to check how your liver and kidneys are working.

Uncommon side effects of axitinib
(Fewer than 1 in 100 people have these)

- An increased risk of getting an infection from a drop in white blood cells (Neutropenia)
- Reversible Posterior Encephalopathy Syndrome (a rare brain disorder which may cause high blood pressure, headache, seizures, vision problems or blindness, dizziness and confusion). It may be reversible if recognised and treated promptly. If you develop any of these symptoms, you should contact your doctor or other healthcare professional immediately.
- A split in the wall of the bowel (bowel perforation) is very rare but is a serious side effect if it happens.

## 12. What should I avoid whilst taking part in the study?

The following should be avoided whilst taking axitinib as they may increase the chance of side effects;

- Do not consume grapefruit or grapefruit juice
- Do not take the following medications;
  - Ketoconazole & Itraconazole (used to treat fungal infections).
  - Clarithromycin, Erythromycin or Telithromycin (used to treat bacterial infections).
  - Atazanavir, Indinavir, Nelfinavir, Ritonavir or Saquinavir (used to treat HIV infections).
  - Nefazodone (used to treat depression).

The following should be avoided whilst taking axitinib as they may reduce the effectiveness of axitinib;

- Do not take the following medications;
  - Rifampicin, Rifabutin or Rifapentin (used to treat tuberculosis).
  - Dexamethasone (steroid medicine)
  - Phenytoin, Carbamazepine or Phenobarbital (anti-eplieptics to stop seizures and fits).
  - St John's wort (herbal product used to treat depression).

You should take special care whilst driving and speak to your doctor if you experience dizziness or feel tired whilst taking axitinib.

## 13. Harm to the unborn child

You will only have started in the study if you have completed a pregnancy test which gave a negative result. However, the medication in this study might harm an unborn child; therefore contraception should be used throughout the duration of the trial. If you think there is any chance you may be pregnant, or your partner has become pregnant it is important to let your study doctor or trials nurse know immediately.

## 14. What will happen if I don't want to continue with the study?

If you agree to take part and then change your mind you can choose to withdraw at any time and you do not have to give a reason. Your decision will not affect the standard of care you receive. However, if you were to withdraw, we would like your permission to continue to collect information on your progress that is routinely recorded in your medical records. This is so that the overall quality of the study is not impaired.

## 15. What if I have to stop participating in the study for another reason?

Your study doctor may decide that continued participation in the study is no longer in your best interest and you may be withdrawn.

For example, you may be withdrawn if the study medication is not working. Your doctor will be able to tell this from the MRI or other scans that you will have during the study. If this is the case, your doctor may decide that you should stop taking the study medication.

You may also be removed from this study by your doctor if you are unable to follow the study schedule, if you develop other medical problems or conditions that would interfere with the study, or if the study procedures are found to be unsafe for you. If you are withdrawn, we

would still like your permission to collect information on your progress that is routinely recorded in your medical records.

You may also be withdrawn if the study is stopped early for any reason. If this does happen, your doctor will discuss treatment options with you.

**16. What happens if there is new information about one of the medicines?**

Axitinib has been authorised for use in the treatment of kidney cancer in the UK since 2012. Sometimes during the course of a research project, new information becomes available about the medicines that are being studied. If this happens, your study doctor will inform you and discuss whether you want to or should continue on study treatment.

If you would prefer not to continue on the study, your doctor will make arrangements for your care to continue, and we would still like your permission to continue to collect information on your progress. If you decide that you do wish to continue in the study you may be asked to sign an updated consent form.

**17. What if there is a problem or something goes wrong?**

In the unlikely event that you are harmed by the study drug, indemnity for your hospital is provided by the usual NHS indemnity arrangements.

If you are harmed due to someone's negligence, the hospital in which you were treated would be liable on the part of hospital staff. Your legal rights are not affected by giving your consent to take part in this study.

If you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of the study, the normal National Health Service complaints mechanisms will be available to you.

Your progress will be watched closely and you will be offered whatever assistance is available to help you cope.

**18. Will my participation in the study be kept confidential?**

Yes. Your medical notes (paper or computerised) will, however, need to be seen by authorised members of the research team at your hospital and the Scottish Clinical Trials Research Unit (SCTRU), which is coordinating the study. There is also a possibility that your medical notes will be reviewed by regulatory authorities in the UK.

Medical records of patients are maintained under strict confidentiality as required by law. Confidentiality will be maintained during and after your participation in the study in accordance with the Data Protection Act 1998. Data collected during the study will be stored and analysed by computer. It will be stored for a prolonged period (over 15 years).

If you consent to take part in the study, only limited identifying information will be recorded, including your initials and date of birth. You will be given a unique 'code' or study number when you enrol, which will be written on all forms relating to your participation in the study. These forms will be sent to the SCTRU with information about your treatment and progress in the study.

In the unlikely event that you experience a serious side effect from the study medication we would pass the information to the drug manufacturer (Pfizer). Pfizer may use your data to submit reports to the regulatory authorities outside the European Union where the data

protections laws are not as stringent as the UK. By signing the trial informed consent form, you are authorising this access. Please be aware, however, that all information about you will be treated as strictly confidential and nothing that might identify you will be revealed to any unauthorised third party.

Once the study is complete the data collected will be processed and analysed by qualified personnel who will also respect the confidentiality and sensitivity of the data. Data obtained from the study may be archived, published in medical journals and presented at international meetings, even if you are excluded and do not participate in the study. In such cases your name will not be used so that confidentiality can be maintained. Your medical information may be held and processed on a computer.

By consenting to participate in this study you understand that all data derived from this study or from the specimens or samples obtained from you is the property of the study sponsor. However, you have the right of access to information relating to yourself and if needed, correction of such information. You have certain rights, which may allow you to have access to information held about you, and to object or prevent certain processing of your information if it will cause you damage or distress. You understand that you may obtain access to such information through your study doctor. For further details on your rights and how you can enforce them, you should contact the study doctors at the study centre.

**19. Involvement of GP/family doctor**

We will inform your GP that you are taking part in this study.

**20. Expenses and Payments**

You will not receive any payment if you agree to take part in this study.

**21. What will happen to any samples that I give?**

To verify the initial diagnosis (performed by the pathologist in your hospital), your tumour might be reviewed by a second independent pathologist who will not necessarily be working in the hospital where you receive treatment. In some cases, a sample of your tumour biopsy, removed at the time of establishing the diagnosis or during a surgical procedure that you may undergo, might be used to perform additional examinations necessary to assure the correct diagnosis.

While the samples should be considered a ‘gift’ they may be used for a range of research including commercial research and this research will include studies outside the UK.

**22. Will any genetic tests be done?**

*Yes. Please see the translational research information sheet.*

**23. What will happen to the results of the research study?**

Independent experts will review the progress of the research, and the results will be published in a respected medical journal as soon as there is enough information to be sure the results are reliable. The results may help to decide how to treat kidney cancer in the future. You will not be identified in any report or publication about the study.

Your hospital will inform you when the results are known and will ask if you would like to see them.

## 24. Who is organising and funding the research?

The Sponsor of the trial is the Common Services Agency or more commonly known as the NHS National Services Scotland. The NAXIVA study is being coordinated by the SCTRU, in Edinburgh, which is an NHS National Services Scotland organisation that receives funding from the Scottish government. All treatment is provided by the NHS.

For this trial, Pfizer, the manufacturer of axitinib, have provided this drug free of charge for patients taking part in this study, as well as providing funds to cover the costs of running the study.

Your doctor will not receive any personal financial rewards for including you in this study.

## 25. Who has reviewed the study?

The NAXIVA study has been reviewed by Pfizer worldwide and endorsed by the Clinical Research Committee (CRC) of Cancer Research UK (CRUK).

The NAXIVA study has also been reviewed for compliance with medical and ethical standards and for scientific value. It has been given a favourable ethical opinion by the East of England – Cambridgeshire and Hertfordshire Research Ethics Committee.

## 26. Further information and contact details

Your study doctor or research nurse will be happy to answer any questions you have about this study. You can telephone them on the numbers shown below, or speak to them again when you come to the clinic.

The Patient Advice and Liaison Service (PALS) is also avaliable to you. PALS offers confidential advice, support and information on health-related matters. They provide information on the NHS, the NHS complaints procedure and support groups outside of the NHS. You can ask your doctor/nurse for details of your nearest PALS alternatively you can search online at www.nhs.uk

You will also be given a card with contact numbers on – please keep this card on you at all times in case of emergency.

| | |
|---|---|
| Your Study Doctor is: | |
| Contact Number: | |
| Your Research Nurse is: | |
| Contact Number: | |

Thank you for reading this information sheet and for considering participating in this research study

*[To be printed on hopsital headed paper]*

**INFORMED CONSENT FORM**

**'NAXIVA'- Main study**

**Phase II neoadjuvant study of axitinib for reducing extent of venous tumour thrombus in clear cell renal cell cancer with venous invasion.**

EudraCT No: 2017-000619-17
ISRCTN96273644
IRAS ID: 223309

Principal Investigator Name:
Centre Number:

**Patient Trial Identification Number:** | **N** | | | | |

Please Initial

I confirm that I have read and understood the information sheet dated 22nd September 2017 version 1.2 for the NAXIVA study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the Scottish Clinical Trials Research Unit, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

I agree to my GP being informed of my participation in the NAXIVA study. ☐

I understand my initials, date of birth and CHI/NHS number will be given when I join the study and thereafter I will be identified by a unique subject number. Any information passed to the regulatory authorities, drug manufacturers and organisations providing services on behalf of the sponsor will not identify me by name. ☐

I understand that the information collected about me will be used to support other research in the future, and may be shared anonymously with other researchers ☐

I understand that information held and maintained by the NHS and other central UK NHS bodies may be used to help contact me or provide information about my health status. ☐

I give permission for data collected during the study to be transferred for the purpose of Pfizer regulatory reports to associated researchers outside the European Economic Area. I understand that some countries outside Europe may not have laws which protect my privacy to the same extent as the Data Protection Act in the UK or European Law. However, as stated in the patient information leaflet they have undertaken to treat ☐

my information as confidential

I agree to take part in the NAXIVA study. 

_____ _____
Signature of subject Date of Signature

_____
Printed name of subject (BLOCK CAPITALS)

I confirm that I have explained the nature of this trial to the above named patient and that they have understood the explanation given to them.

_____ _____
Signature of person taking informed consent Date of Signature

_____
Printed name of person taking informed consent (BLOCK CAPITALS)

*1 copy for patient; 1 for researcher (original); 1 to be kept with hospital notes*

**Appendix 5 –Translational Patient Information Sheet and Informed Consent**

*[To be printed on hospital headed paper]*

**PATIENT INFORMATION SHEET – Translational Research**

**'NAXIVA'**

**Phase II neoadjuvant study of Axitinib for reducing extent of venous tumour thrombus in clear cell renal cell cancer with venous invasion**

EudraCT No: 2017-000619-17
ISRCTN96273644
IRAS ID: 223309

PIS Version: 1.1
Version Date: 30th June 2017

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**Invitation Paragraph**
You are being invited to take part in a research study. Before you decide if you want to take part it is important for you to understand why the research is being done, how your information will be used, what the research will involve and the possible benefits, risks and discomforts. Please take time to read the following information carefully and discuss it with your relatives, friends or GP if you wish.

Please ask if there is anything that is not clear or if you would like further information.

**Further Research**

**1. What is translational research?**
Translational research is where findings that have been made in the basic science research laboratory are further developed with the aim of making a change in the treatment of a disease. Translational research is often called 'bench to bedside research'. Translational research often makes use of the samples donated by a patient to better understand and treat the disease.

**2. What is the purpose of the translational research in NAXIVA?**
Within the NAXIVA trial we aim to use translational research to gain a better understanding of kidney cancer and its treatments with the class of medication (tyrosine kinase inhibitors) you will be taking in the study. We aim to identify 'biomarkers' that might allow identification of patients most likely to benefit from the drug given in NAXIVA. We also hope to gain insights into the development of resistance of some cancers to tyrosine kinase inhibitors.
This extra research that may be done with your samples is not designed to specially benefit you. It may have an impact on the choice of specific treatment for this type of kidney cancer for others. By broadening the knowledge about kidney cancer, it may help other patients in your situation.

**3. Why have I been invited to take part?**
Your Oncologist feels that taking extra samples of blood, urine and your tumour would be safe and may help with our understanding of treatment of kidney cancer such as your own.

## 4. Do I have to take part?

No, it is up to you to decide whether or not to take part. If you do choose to take part you will be asked to sign an informed consent form and you will be given a copy to keep together with this information sheet. For your safety your GP will be informed of your participation in this research.

If you do not wish to take part in the translational research you do not have to give a reason. You will not be disadvantaged in any way, and it will not affect the standard of care you receive. This also applies if you initially decide to take part and then change your mind at a later date.

## 5. What will happen to me if I decide to take part?

You will have a biopsy taken of your kidney cancer along with a series of scans, tests and examinations to confirm you are eligible to take part in the NAXIVA study. At the time of biopsy, 4 extra tissue samples will be taken for research use within the NAXIVA trial. These will be done approximately 1 to 4 weeks before you start taking any study medication.
In addition, at the time you have your surgery to remove your cancer (week 9); a tissue sample will be taken from the removed cancer for further analysis.
The extra tissue will be sent and stored at the University of Cambridge Laboratory.

You will have routine blood and urine samples taken throughout the NAXIVA study to monitor your progress. At each follow up prior to your surgery and at your final clinic visit (12 weeks post surgery) you will have 2 extra blood samples taken and be asked to provide 1 extra urine sample. These further tests will be used for side research to the NAXIVA study. If you agree to provide extra blood and urine samples, they will be sent and stored at the University of Cambridge Laboratory. One of your blood samples will be sent outside the European Union to Eve Technologies in Canada. Eve Technologies specialise in a specific analysis of blood and are currently the only institution in the world that can carry out this analysis. Your blood sample will be fully anonymised and will not be linked to you in anyway.

This research will not have an effect on the standard of your medical care.

Trial Visit Schedule

| | Screening | Visit 1 | Visit 2 | Visit 3 | Visit 4 | Visit 5 | Visit 6 | Visit 7 | Visit 9 |
|---|---|---|---|---|---|---|---|---|---|
| | 1-4 weeks prior to study enrolment | Week 1 | Week 3 | Week 5 | Week 7 | Week 9 | Week 9 | 6 weeks post surgery | 12 weeks post surgery |
| Biopsy | X | | | | | | | | |
| Blood & Urine samples | | X | X | X | X | X | | | X |
| Surgery | | | | | | | X | | |

## 6. What will I be asked to do?

If you agree to take part in this sub-study, the tissue and blood samples will be taken and the urine sample collected at standard NAXIVA trial clinic visits.

## 7. What will happen to any samples that I give?

If you agree to take part in this translational research, and with your permission, we will ask the pathologist at the laboratory in your hospital to send the extra tissue, blood and urine samples to the University of Cambridge laboratory to carry out the research.

In addition, 1 of the blood samples you supply during the trial will be sent outside the European Union for processing and analysis. This sample will be fully anonymised and will not be linked to you in anyway.

At the end of the research your samples will be sent to a central tissue bank for continued storage. These samples may be used in future research which may be able to give us a better understanding of which treatments are best for which patients.

**8. What are the possible disadvantages and risks of taking part?**

The tissue and blood samples for research will be taken at the time of routine samples needed for your clinical care, i.e. with no extra passes of the biopsy or blood taking needle. However, there can be some discomfort from the needle used for biopsy of the kidney lump or blood taking and bruising or bleeding afterwards. There is a small chance of infection which is minimised by taking the samples under sterile conditions.

**9. What if there is a problem or something goes wrong?**

In the unlikely event that you are harmed during the taking of the extra samples indemnity for your hospital is provided by the usual NHS indemnity arrangements.

If you are harmed due to someone's negligence, the hospital in which you were treated would be liable on the part of hospital staff. Your legal rights are not affected by giving your consent to take part in this research.

If you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of the translational research, the normal National Health Service complaints mechanisms will be available to you.

**10. Will my participation in the translational research be kept confidential?**

Yes. Your medical notes (paper or computerised) will, however, need to be seen by authorised members of the research team at your hospital and the Scottish Clinical Trials Research Unit (SCTRU), which is coordinating the study. There is also a possibility that your medical notes will be reviewed by regulatory authorities in the UK.

Medical records of patients are maintained under strict confidentiality as required by law. Confidentiality will be maintained during and after your participation in the study in accordance with the Data Protection Act 1998. Data collected during the study will be stored and analysed by computer. It will be stored for a prolonged period (over 15 years).

If you consent to take part in the translational research, only limited identifying information will be recorded, including your initials and date of birth. You will be given a unique 'code' or study number when you enrol, which will be written on all specimens relating to your participation in the study.

One of your blood samples will be sent outside the European Union to Canada for analysis. Some countries outside the European Union may not have laws which protect your privacy to the same extent as the Data Protection Act in the UK or European Law.
In this instance your sample would be fully anonymised (labelled 1-100) and will have no link to you in anyway.

Once the study is complete the data collected will be processed and analysed by qualified personnel who will also respect the confidentiality and sensitivity of the data. Data obtained from the study may be archived, published in medical journals and presented at international meetings, even if you are excluded and do not participate in the study. In such cases your name will not be used so that confidentiality can be maintained.

By consenting to participate in the translational research you understand that all data derived from the specimens or samples obtained from you is the property of the study sponsor. However, you have the right of access to information relating to yourself and if needed, correction of such information. You have certain rights, which may allow you to have access to information held about you, and to object or prevent certain processing of your information if it will cause you damage or distress. You understand that you may obtain access to such information through your study doctor. For further details on your rights and how you can enforce them, you should contact the study doctors at the study centre.

**11. Involvement of GP/family doctor**

We will inform your GP that you are taking part in this study.

**12. Expenses and Payments**

You will not receive payment if you agree to take part in this study.

**13. What will happen to the results of the translational research?**

The results will be published in a respected medical journal and may help to decide how to treat kidney cancer in the future. You will not be identified in any report or publication about the study. Following the open access ethos, we will make the 'raw data' from experiments available to other researchers. This data will not identify you in anyway.

Your hospital will inform you when the results are known to ask if you would like to see them.

**14. Will any genetic tests be done?**

Genetic analysis of your tumour tissue or blood may be undertaken. This will be to evaluate potential biomarkers (measurable indicators of the severity or presence of some disease state) in kidney cancer.

**15. Further information and contact details**

Your study doctor or research nurse will be happy to answer any questions you have about any aspect of the study. You can telephone them on the numbers shown below, or speak to them again when you come to the clinic.

The Patient Advice and Liaison Service (PALS) is also avaliable to you. PALS offers confidential advice, support and information on health-related matters. They provide information on the NHS, the NHS complaints procedure and support groups outside of the NHS. You can ask your doctor/nurse for details of your nearest PALS alternatively search online at www.nhs.uk

You will also be given a card with contact numbers on – please keep this card on you at all times in case of emergency.

| | |
|---|---|
| Your Study Doctor is: | |
| Contact Number: | |
| Your Research Nurse is: | |
| Contact Number: | |

Thank you for reading this information sheet and for considering participating in this research study.

*[To be printed on hospital headed paper]*

**INFORMED CONSENT FORM – Translational Research**

**'NAXIVA'**

**Phase II neoadjuvant study of Axitinib for reducing extent of venous tumour thrombus in clear cell renal cell cancer with venous invasion**

EudraCT No: 2017-000619-17
ISRCTN96273644
IRAS ID: 223309

Principal Investigator Name:
Centre Number:

**Patient Trial Identification Number:** | **N** | | | | |

Please Initial

I confirm that I have read and understood the information sheet dated 30th June 2017 version 1.1 for the NAXIVA translational research and I have been given the opportunity to consider the information, discuss the research, ask questions and have had these answered satisfactorily. ☐

I understand that my participation in the translational studies is entirely voluntary and that I am free to withdraw at any time, without giving reason, and without my medical care or legal rights being affected. ☐

I understand that extra biopsy samples of my kidney tumour tissue will be taken during tumour biopsy. In addition I understand that excess tissue, will be taken from my kidney tumour removed at surgery. The results of these analyses will not be made available to me or the team looking after me as they are purely for research purposes. ☐

I understand that extra blood samples will be taken from me at every clinic visit before and after surgery. The results of these analyses will not be made available to me or the team looking after me as they are purely for research purposes. ☐

I understand that extra urine samples will be taken from me at every clinic visit before and after surgery. The results of these analyses will not be made available to me or the team looking after me as they are purely for research purposes. ☐

I give permission for samples collected during the study to be transferred for the purpose of analysis to associated researchers outside the European Economic Area. I understand that some countries outside Europe may not have laws which protect my privacy to the same extent as the Data Protection Act in the UK or European Law However, as stated in the patient information leaflet they have undertaken to treat my information as confidential ☐

I consent to take part in the translational research within the NAXIVA study. ☐

_____ _____
Signature of subject Date of Signature

_____
Printed name of subject (BLOCK CAPITALS)

I confirm that I have explained the nature of this trial to the above named patient and that they have understood the explanation given to them.

_____ _____
Signature of person taking informed consent Date of Signature

_____
Printed name of person taking informed consent (BLOCK CAPITALS)

*1 copy for patient; 1 for researcher (original); 1 to be kept with hospital notes*

## Appendix 6 – Translational Research Studies

### 1. Introduction

A key aim of this study is to evaluate changes in multiple molecular factors in relation to tumour response.
The expression of these putative biomarkers of renal cancer response will be evaluated in tissue donated before and following treatment to define therapeutic response to treatment. In addition, novel techniques (including immunophenotyping and metabolomics analysis) will be applied to samples to gain a better fundamental understanding of the effect of TKIs on metabolites, immune function, proteomics and also to assess the potential for these and other markers (such as ctDNA) for predicting or tracking response to drugs.
Analysis of tissue for such studies will be performed at the Stewart Lab, Academic Urology Group, Department of Surgery, University of Cambridge and collaborators within the Cambridge Renal Cancer (CamRenCan) collaborative.

### 2. NAXIVA laboratory manual

Detailed instructions for sample collection, processing, labelling, storage and transportation will be provided in the NAXIVA laboratory manual which is available from the Scottish Clinical Trials Research Unit (SCTRU) and should be referred to in conjunction with this protocol. It is essential that these protocols are followed to enable reproducible high quality translational analyses.

### 3. Tissue Acquisition and Processing at site

#### 3.1. Baseline Primary tumour tissue sample

Image guided percutaneous biopsy of the primary tumour should be performed prior to starting treatment. Five tissue cores from one needle puncture should be obtained: two cores should be formalin-fixed and sent for paraffin-embedding by the local pathologist; one being retained by the site and used to establish a histological diagnosis whilst the other should be transferred to The University of Cambridge (this can be repatriated if a histological diagnosis cannot be obtained for whatever reason). One of the remaining cores should be snap frozen in liquid nitrogen within 30 minutes after biopsy and stored at -80°C. The remaining 2 biopsies should be placed in 5ml media (as provided to the sites by The University of Cambridge) immediately after biopsy, stored at 4°C, and couriered to The University of Cambridge as soon as possible after biopsy (maximum within 24 hours of biopsy). These samples will be processed for immunophenotyping. Remaining biopsies should be transferred to the University of Cambridge on dry ice (or room temperature for formalin-fixed paraffin-embedded (FFPE) blocks).
**In the event of less than 5 biopsy cores being available please refer to the NAXIVA laboratory manual which is available on request from SCTRU for prioritisation of tissue processing.**

#### 3.2. Nephrectomy specimen

Nephrectomy specimens should be dissected by the local pathologist to obtain sufficient material for routine diagnostic purposes. Following this, multiregion sampling of tumour tissue, venous tumour thrombus and normal kidney should be undertaken with an accompanying photo or diagram. These tissues should be snap frozen in liquid nitrogen within 1 hour after the blood supply to the kidney has been interrupted and stored at -80°C at the local trial centre until transferred to the University of Cambridge on dry ice. At the same time, samples should be taken from immediately adjacent to the above samples and should be immediately formalin-fixed and paraffin-embedded and stored at room temperature at the local trial centre until transferred to the University of Cambridge at room temperature.

Additionally, approximately 1cm$^3$ of tumour, venous thrombus, and normal kidney should be placed in 5ml media (as provided to the sites by The University of Cambridge) within 1 hour after the blood supply to the kidney has been interrupted, stored at 4°C, and couriered to The University of Cambridge as soon as possible after biopsy (maximum within 24 hours of biopsy). These samples will be processed for immunophenotyping, and primary culture of malignant and stromal cells. Samples should also be taken from immediately adjacent to the above samples and should be immediately formalin-fixed and paraffin-embedded and stored at room temperature at the local trial centre until transferred to the University of Cambridge at room temperature.
**Trial centres should notify SCTRU one week in advance of any planned surgery.**

### 3.3. Paraffin Embedded Tissue Sections

Paraffin embedded core biopsies that are no longer needed by the local pathologist for diagnostic purposes may be requested for use in translational work, following discussion with the local site consultant pathologist.

### 3.4. Blood and Urine Processing

Patients should be fasted for 6 hours prior to urine sampling. Therefore, it is suggested that blood and urine sampling is performed as early in the morning as clinically feasible, and the times of the patient's last meal and last axitinib should be recorded.

#### 3.4.1. Blood Samples

Blood - Venous blood for biomarker analysis will be collected in ethylenediaminetetraacetic acid (EDTA) tubes. **Two** 10ml tubes will be collected on day 1 week 1 prior to starting axitinib treatment, **two** tubes at the week 3 clinic visit**, two** tubes at the week 5 clinic visit, **two** tubes at the week 7 clinic visit, **two** at time of nephrectomy and **two** tubes at 12 weeks post surgery follow up. All blood samples must be processed locally. Blood samples must be centrifuged and processed for whole blood and plasma within 1 hour of blood sampling, and within 4 hours of blood sampling for collection of buffy coat and peripheral blood mononuclear cells (PBMCs) from the remaining blood. Detailed instructions for blood processing are contained in the NAXIVA laboratory manual. From these two EDTA tubes the following samples must be collected:

**Whole blood (WB) – all centres:** Immediately after blood sampling gently invert the tubes 8-10 times to mix and leave tubes upright prior to further processing. From one of EDTA tubes, remove 1ml whole blood and place into a screw cap cryovial (labelled with 'WB, patient's trial number and the date). Snap freeze the tube in liquid nitrogen and store at -80°C at the trial centre until transfer to University of Cambridge on dry ice.

**Plasma (P) – all centres:** This step **must be completed within 1 hour** of blood sampling. Following transfer of whole blood from one vacutainer, spin both vacutainer tubes at 1600g for 10 min at room temperature using a "swing-out" rotor. Transfer 1ml aliquots of plasma to sterile 2ml micro-tubes (non-screw capped RNase-free tubes can be used at this stage). Take care to avoid any buffy coat layer in this step. Centrifuge the plasma aliquots in a bench top centrifuge at 14,000rpm for 10 minutes at 4°C to pellet any remaining cellular debris. Carefully transfer 1ml aliquots of supernatant to labelled (P/patient's trial number/date) sterile 1.8ml screw-capped cyrovials, and discard the pellet. Store the original vacutainers with remaining blood at 4° C for collection of buffy coat or Peripheral Blood Mononuclear Cells (PBMCs). Aim to collect 4 cryovials with roughly even amounts of plasma in each. Store at -80°C at the trial centre until transfer to University of Cambridge on dry ice.
**All plasma samples MUST be double-spun using the NAXIVA protocol above to allow down-stream translational assays to be performed.**

**Buffy coat (BC)**
**For collection ONLY by sites NOT processing samples for PBMCs (as this step removes the PBMCs):** Following removal of whole blood and plasma from the vacutainers, remove the buffy coat with about 100ul of plasma taking care not to lift the red cells. Optional: If it is difficult to cleanly separate the buffy coat layer, combine the buffy coats from both vacutainers in a small Eppendorf tube/micro centrifuge tube and centrifuge at 10,600g for 2 minutes. Then aliquot into 1 x labelled cryovial (BC/patient's trial number/date). Store at -80°C at the trial centre until transfer to University of Cambridge on dry ice.

**Peripheral blood mononuclear cells (PBMCs)**
**Do NOT collect buffy coat if processing blood for PBMCs as buffy coat contains PBMCs resulting in very low yield using this protocol**.
This method requires the use of SepMate tubes, ficoll, and 'freezing mix' which will be provided to selected sites by the SCTRU as part of the sample collection kits. All processing steps (including removal of plasma from vacutainers) MUST be carried out under sterile conditions eg. HEPA filtered CL2-certified tissue culture facilities, if blood samples are being used to isolate PBMCs.

1. Ideally this step must be completed within 4 hours of blood sampling, but certainly completed same day. If unable to process samples immediately after plasma extraction, store samples at 4°C.
2. After removing plasma dilute the remaining blood in the tube 1:1 with pre-warmed (room temperature) sterile phosphate buffered saline (PBS) (if a full 10ml EDTA tube was taken from the patient the volume of PBS to add will be approximately 4ml).
3. Mix tube well by inverting 3 times.
4. Keeping the SepMate tube upright, pipette 15ml Ficoll through the central hole of the insert in the SepMate tube. The top of the Ficoll will come to ABOVE the level of the insert. Air bubbles may be seen under the insert and are reduced by pipetting the Ficoll more slowly through the insert, but don't affect the cell separation.
5. Quickly pipette the blood/PBS mix down the side of the SepMate tube.
6. Centrifuge tubes at 1200g (2800 rpm) for 10 minutes at room temperature (using normal brake settings ie brake on).
7. Remove tubes from centrifuge and collect the mononuclear cells by inverting the SepMate tube into a 50ml Falcon tube in one smooth movement. Do not hold the SepMate tube inverted for >2 seconds or the red cells (which may be seen on the surface of the SepMate insert before inversion in addition to the bottom of the tube) start to dribble through the insert and will contaminate the mononuclear cells.
8. Dilute mononuclear cells to 45ml with pre-warmed PBS.
9. Centrifuge tubes at 1200g (2800 rpm) for 10 minutes at room temperature (normal brake settings) to wash the cells.
10. Discard supernatant (carefully as cell pellet may be slimy).
11. Resuspend cell pellet in 2ml 10% dimethylsulfoxide in fetal calf serum ('freezing mix') and freeze 4 labelled (PBMC/patient's trial number/date) cryovials of PBMCs (aliquot equal volumes into each cryovial and record volumes).
12. Immediately place at -80°C (in a pre-cooled (4°C) Mr Frosty/ similar container). NB check isopropanol level beforehand. Store at -80°C for up to a week, and then transfer to liquid nitrogen until transfer on dry ice to University of Cambridge.

### 3.4.2. Urine Samples– all centres

Urine samples will be collected on day 1 week 1, week 3, week 5, week 7 at nephrectomy and 12 week post surgery follow-up.
15ml of urine (ideally after patient fasted for 6 hours, fasting time to be noted) should immediately be stored at -80°C at trial centres until transfer to University of Cambridge on dry ice.

In addition, 30ml urine in a falcon tube should have EDTA added immediately to prevent nuclease degradation (for cfDNA analysis). Add 600ul of 0.5M EDTA and gently invert 8-10 times. Centrifuge at 3000g for 10 minutes within 1 hour of collection to remove cellular debris. Transfer 3.6ml aliquots of supernatant to fresh sterile 4ml cryotubes. Retain cell pellet, suspended in 1ml urine.
Each specimen container should be labelled as specified in the NAXIVA laboratory manual. Labels will be supplied by the SCTRU as part of the sample collection kits.

## 4. Storage and Collection of Samples

Blood, urine and tissue samples should be stored at local trial centres in a -80°C freezer and transferred in batches as detailed in the NAXIVA laboratory manual to the University of Cambridge on dry ice unless part of the fresh tissue for immunophenotyping/ cell culture study. Samples which are part of the fresh tissue for immunophenotyping/ cell culture study MUST be stored at 4°C and couriered to University of Cambridge in insulated polystyrene containers with ice packs in situ as soon as possible after collection (certainly within 24 hours of collection but ideally same day). Further details for transfer of these samples can be found in the NAXIVA laboratory manual.

### 4.1. Translational sample Schedule

| Visit/Assessment | Within 28 days prior to registration | Week 1 | Week 3 | Week 5 | Week 7 | Nephrectomy | 12 weeks post surgery follow up |
|---|---|---|---|---|---|---|---|
| Percutaneous image guided biopsy of non-necrotic region of primary renal tumour (5 cores, 2 to be sent to pathology for formalin-fixation and paraffin embedding of which 1 to be transferred to University Cambridge; 1 core to be snap frozen in liquid nitrogen and stored at -80°C; 2 to be placed in tubes containing media and couriered within 24 hours to University of Cambridge) | X | | | | | | |
| Nephrectomy tissue | | | | | | X | |
| 2 EDTA tube blood samples for whole blood, double-spun to obtain plasma, buffy coat and PBMCs at selected sites (samples to be stored at -80°C) | | X | X | X | X | X | X |
| Urine sample (1 container to be stored at -80°C, 1 sample EDTA treated and spun sample at -80°C) | | X | X | X | X | X | X |

### 4.2. Sample Tracking

Sites will be required to record the collection of participants' samples by emailing a sample reporting form to the central lab at the University of Cambridge and SCTRU. SCTRU will provide courier account details for sites to organise transport of the samples.

#### 4.2.1. Tracking of baseline samples for ineligible patients

In order to minimise invasive procedures, patients will be consented for the trial prior to obtaining both the standard diagnostic biopsy sample and baseline translational study research biopsies. Patients will be asked to provide consent for the research samples to be sent to the University of Cambridge should histological examination of the diagnostic sample show that the patient is ineligible for NAXIVA. Collection of these samples should be notified to the SCTRU by emailing: NSS.NAXIVA@nhs.net

The following information will be required:

- Centre name
- Confirmation of written informed consent
- Trial ID
- Patient's date of birth

This notification will allow these samples to be tracked by the University of Cambridge and SCTRU.

### 4.3 Expedited reporting of fresh tumour collection at biopsy and planned nephrectomy

All participating sites must notify SCTRU in advance of the date of planned biopsy or nephrectomy.
Centres should notify SCTRU of the biopsy or nephrectomy, by completing the NAXIVA expedited notification of tissue collection form (included within the NAXIVA laboratory manual) and emailing it to: NSS.NAXIVA@nhs.net

SCTRU will then notify University of Cambridge (Mr Grant Stewart and Dr Sarah Welsh) to expect samples.

### 4.4 Tissue Sample processing at the University of Cambridge

All samples will be catalogued in an electronic database at the University of Cambridge. The time from core biopsy collection to snap freezing and from ischaemia to snap freezing for nephrectomy specimens will be recorded on a database. All tissues will be stored in an alarmed and locked -80°C freezer (or liquid nitrogen tank for PBMCs and dissociated tumour samples) which are dedicated to the storage of clinical trial patient samples.

#### 4.4.1 Blood Specimens (Whole blood, Plasma, Buffy coat, PBMC)

Analysis of whole blood, plasma, buffy coat, and PBMCs for translational studies will be conducted at the University of Cambridge. Plasma will be analysed for biomarkers (including metabolites, circulating tumour (ct) DNA, and cytokines). DNA will be extracted from whole blood/ or buffy coat and PBMCs for sequencing of host DNA. PBMCs will be immunophenotyped using CyTOF.

#### 4.4.2. Urine Specimens

Analysis of urine samples will be conducted at University of Cambridge. Urine will be analysed for biomarkers (including metabolites, circulating tumour (ct) DNA, and cytokines).

#### 4.4.3 Core Biopsy and Nephrectomy Specimens

Processing and analysis of core biopsy, nephrectomy and surgical specimens will be conducted at the University of Cambridge. Dissociation of fresh tumour, venous thrombus and normal kidney samples will be performed to separate malignant cells from associated

stromal and immune components, and immunophenotyping will be performed using CyTOF. Cell lines (tumour, stomal, lymphocyte) will be generated if sufficient tissue allows. RNA and DNA from each will be extracted from fresh-frozen samples according to Good Laboratory Practice standards. Immunohistochemistry and immunofluorescence staining will be performed on FFPE samples.

## 5. Analysis of Biosamples

Biosample analysis aims to correlate changes in biomarkers with toxicity and response to axitinib treatment.
The techniques below may be used to explore this endpoint and are based on the current literature. Equivalent novel methods and technologies might be used at the time of analysis.

### 5.1. Metabolomics

It is well established that there is a strong metabolic basis to ccRCC. The presence of the Warburg effect has revealed several novel targets for treatment or tumour surveillance. Dr Frezza is an expert in metabolomics, working mainly on inherited forms of kidney cancer. Using LC-MS, metabolites will be assayed in urine, blood and tissue across the various time points of the NAXIVA study to assess the use of these molecules in patients with IVC VTT and with Axitinib therapy. This work will be discovery in nature assessing alterations in metabolic profile within protein from tumour, plasma and urine at sequential time points within the NAXIVA study.

### 5.2. Circulating Tumour DNA

In view of intratumoural heterogeneity, the use of tumour tissue for the development of prognostic and predictive biomarkers in ccRCC is challenging. Circulating tumour DNA (ctDNA) has been established to represent the tumour biology and may be superior to tissue as heterogeneity will be negated. Variant analysis will be undertaken on multi-region sampled issue using exome and shallow whole genome sequencing. A custom capture panel for targeted higher-depth (and higher sensitivity) sequencing of plasma and urine samples will be developed for each patient to assess the feasibility of tracking circulating DNA molecules that harbour somatic mutations. The end-point of this analysis will be to determine the ability to identify variants in both tissue and plasma and the change in ctDNA profiles from baseline to treatment phase to posttreatment phase which may be informative regarding markers of tumour response and minimal residual disease. Further validation studies of any positive findings will be undertaken separately.

### 5.3. Proteomics

Formalin fixed paraffin embedded biopsy and nephrectomy tissue will be utilised in an automated quantified immunohistochemical based analysis of putative markers of response to tyrosine kinase inhibitors. In addition to Ki67 measurement of effect of Axitinib on proliferation, other markers of response to tyrosine kinase inhibitors will be evaluated. In recent work, it has been established that modulation of CA9 protein levels occurs following sunitinib therapy in metastatic clear cell RCC (ccRCC); this effect will be validated using patient samples from NAXIVA. The outcome of this work will be to establish a link between expression of these proteins and reduction in height of the VTT with axitinib. If a correlation is established these proteins will be helpful as surrogate translational endpoints in future ccRCC neoadjuvant studies.

### 5.4. Immune studies

It is known that RCC is a typical immunogenic tumour with immune mechanisms known to play an important role in its natural history. Recent studies have also shown that TKI treatment can influence the immune system and, importantly, changes in the immune response may affect response and survival. The immune studies aim to identify immune- and cytokine-based signatures in PBMCs, plasma and urine which predict response to

axitinib treatment in RCC patients using CyTOF and luminex-based cytokine assays. These studies will also provide important information about the effect of axitinib on the immune system in RCC, and vice versa, including providing comprehensive analysis of how peripheral immune cell populations and cytokines vary during treatment with axitinib, and whether they correlate with other markers of response and resistance in RCC patients. Importantly, it will also provide essential immunological information to inform biologically driven, rational, scheduling of future studies examining combinations of axitinib and immunotherapy.

**Appendix 7– The Principles of ICH Good Clinical Practice**

1. Clinical trials should be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with GCP and the applicable regulatory requirement(s).
2. Before a trial is initiated, foreseeable risks and inconveniences should be weighed against the anticipated benefit for the individual trial subject and society. A trial should be initiated and continued only if the anticipated benefits justify the risks.
3. The rights, safety, and well-being of the trial subjects are the most important considerations and should prevail over interests of science and society.
4. The available nonclinical and clinical information on an investigational product should be adequate to support the proposed clinical trial.
5. Clinical trials should be scientifically sound, and described in a clear, detailed protocol.
6. A trial should be conducted in compliance with the protocol that has received prior institutional review board (IRB)/independent ethics committee (IEC) approval/ favourable opinion.
7. The medical care given to, and medical decisions made on behalf of, subjects should always be the responsibility of a qualified physician or, when appropriate, of a qualified dentist.
8. Each individual involved in conducting a trial should be qualified by education, training, and experience to perform his or her respective task(s).
9. Freely given informed consent should be obtained from every subject prior to clinical trial participation.
10. All clinical trial information should be recorded, handled, and stored in a way that allows its accurate reporting, interpretation and verification.
11. The confidentiality of records that could identify subjects should be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).
12. Investigational products should be manufactured, handled, and stored in accordance with applicable good manufacturing practice (GMP). They should be used in accordance with the approved protocol.
13. Systems with procedures that assure the quality of every aspect of the trial should be implemented.